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# ROYAL SOCIETY.

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## REPORTS TO THE MALARIA COMMITTEE.

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### SEVENTH SERIES.

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#### CONTENTS.

Reports from Messrs. STEPHENS and CHRISTOPHERS, India.

1. The Classification of Indian Anopheles into Natural Groups.
  2. The Relation of Species of Anopheles to Malarial Endemicity.
  3. The Relation of Species of Anopheles to Malarial Endemicity—Further Report.
  4. An Investigation into the Factors which determine Malarial Endemicity.
  5. Note on Bodies in Salivary Glands of Anopheles, etc.
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6. On a convenient Terminology for the Various Stages of the Malaria Parasite. By Professor RAY LANKESTER, F.R.S.
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LONDON:  
HARRISON AND SONS, ST. MARTIN'S LANE,  
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1902.

*Price Three Shillings.*

AUGUST 15, 1902.

Fb 11.87

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# REPORTS, &c., FROM MESSRS. STEPHENS AND CHRISTOPHERS, INDIA.

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“The Classification of Indian Anopheles into Natural Groups.”

By J. W. W. STEPHENS, M.D. Cantab., and S. R. CHRISTOPHERS, M.B. Vict. Received February 6, 1902.

[PLATES 1—4.]

We would take this opportunity of acknowledging our indebtedness in all our Indian work to Captain S. P. James, I.M.S., who was attached to the Malaria Commission by the Indian Government. He has throughout assisted us in the examination of blood films, dissections, and other incidental work. We are especially indebted for much help in the differentiation of the species of Indian anopheles.

Since it seemed possible to us that the question of species of Anopheles might be an important factor in determining variations in the distribution of malaria, we have investigated the appearance of the adult insect, the character of the ovum and larva, and the habits of each species encountered by us in India.

The result of this investigation has been to show that a subdivision of the genus *Anopheles* into several natural groups can be made.

The members of some of these groups very closely resemble one another in almost every particular, especially in the habits of the species.

## *The Adult Insects.*

The species of *Anopheles* noted in India are at present the following:—

- |  |   |
|--|---|
| (1) <i>A. barbirostris</i> (Van der Wulp). | (9) <i>A. maculatus</i> (Theobald).           |
| (2) <i>A. Sinensis</i> (Wied.).            | (10) <i>A. Theobaldi</i> (Giles).             |
| Sub-sp. <i>nigerrimus</i> (Theobald).      | (11) <i>A. Rossii</i> (Giles).                |
| Syn. <i>A. nigerrimus</i> (Giles).         | (12) <i>A. Stephensii</i> (Liston and James). |
| (3) <i>A. culicifacies</i> (Giles).        | Syn. <i>A. metaboles</i> (Theobald).          |
| (4) <i>A. Listonii</i> (Giles).            | (13) <i>A. pulcherrimus</i> (Theobald).       |
| (5) <i>A. Christophersi</i> (Theobald).    | (14) <i>A. Turkhudi</i> (Liston).             |
| (6) <i>A. Indicus</i> (Theobald).          | (15) <i>A. Lindsayi</i> (Giles).              |
| (7) <i>A. Jamesii</i> (Theobald).          | (16) <i>A. Gigas</i> (Giles).                 |
| (8) <i>A. fuliginosus</i> (Giles).         |   |
| Syn. <i>A. Jamesii</i> (Liston).           |   |

Many of these species are obviously closely related, and differ from one another only in minute particulars. Considering only the adult female, the species known to us may be grouped as follows:—

Group I. Large black species with broad scales upon many of the veins of the wings.

*A. barbirostris*.

*A. Sinensis*, sub-sp. *nigerrimus*.

Group II. More or less dark species with markedly banded legs of white tarsi.

*A. maculatus* (Theobald).

*A. Theobaldi* (Giles).

*A. Jamesii* (Theobald).

*A. fuliginosus* (Giles).

*A. pulcherrimus* (Theobald).

Group III. Very small species with dark, unbanded legs; wings with veins, for the most part covered with dark scales.

*A. Indicus* (Theobald).

*A. culicifacies* (Giles).

*A. Listonii* (Giles).

*A. Christophersi* (Theobald).

*A. Turkhudi* (Liston).

Group IV. Light brown species with the wings for the most part covered with light scales.

*A. Rossii* (Giles).

*A. metaboles* (Theobald).

Group V. *A. Lindesayi* (Giles).

#### *Variations in the Characters of the Adult Insects of each Species.*

Many species of anopheles exhibit considerable variation in—

1. The size of the insect. Especially in *A. Rossii* and *A. Stephensi*, but also to a less degree in *A. culicifacies* and other species, we have noted very great differences in the size of individuals.

This we think is due to a lack of nutrition of the larvæ, as larvæ taken from foul and overcrowded puddles most frequently hatch out as small specimens.

2. The wing markings. A degree of variation occurs in the extent of vein covered by each area of dark scales.

In *A. Stephensi* very considerable variations are seen in the first longitudinal vein, so that the second large wing-spot is most variable in form. In this species the wings on the same insect sometimes differ.

In *A. Rossii* an extra dark area is frequently added to the T-spot,\* and the first and second costal spots are sometimes confluent.

3. The leg markings may be faint or well marked in the same species.

*The Ovum*.—The ovum of each species shows marked specific characters.

Three very distinct types of ovum have been seen by us—

Type 1. Ova having the upper surface very narrow, with the lateral floats not touching the margin. (See Plate 1, fig. 1.)

The species with ova of this type are—

*A. barbirostris*.

*A. culicifacies*.

*A. Sinensis*, sub-sp. *nigerrimus*.

*A. Christophersi*.

Type 2. Ova having a more or less broad upper surface, with the lateral floats touching the margin. (See Plate 1, figs. 2, 3, and 4.)

Species having ova of this type are—

*A. Rossii* (Giles).

*A. fuliginosus* (Giles).

*A. pulcherrimus* (Theobald).

*A. metaboles* (Theobald).

Type 3. Ova with no floats, and with upper surface rudimentary. (See Plate 1, fig. 5.)

One species only has ova of this type, viz. :—

*A. Turkhudi* (Liston).

Species having ova of the first type have in all cases been species breeding in either open natural waters or running streams.

Species with ova of the second type are in general found breeding in pools.

2. *The Larva*.—The larva of each species also show more or less marked specific differences.

Four groups of larvæ may be distinguished readily by variations in certain hairs, called by us frontal hairs (see 'Report to Malaria Committee,' No. VI).

Type 1. Larvæ with the external pair of frontal hairs converted into a cockade-like tuft. (See Plate 2, figs. 6, 6A, and 6B.)

Species having larvæ of this type are—

*A. barbirostris*.

*A. Sinensis*, sub-sp. *nigerrimus*.

Larvæ of this type also have a large branched hair upon the antenna, and the leaflets of the palmate hairs differ markedly from all other larvæ. (See Plate 4, fig. 15A.)

\* Especially seen in the male.—(F. V. T.).

Type 2. Larvæ with the external frontal hairs branched, but not developed into tufts. (See Plate 2, figs. 7 and 9.)

*A. fuliginosus.*

*A. pulcherrimus.*

Type 3. Larvæ with the external pair of frontal hairs simple and unbranched, and with palmate hairs on every abdominal segment, and on the thorax. (See Plate 2, fig. 10, and Plate 3, fig. 10A.)

*A. culicifacies.*

*A. Listonii.*

*A. Christophersi.*

Type 4. Larvæ with the external pair of frontal hairs simple and unbranched, but with no developed palmate hairs on thorax or first abdominal segment. (See Plate 3, figs. 11, 12.)

*A. Rossii.*

*A. metaboles.*

Type 5. Larvæ with two large additional hairs placed behind those already mentioned. Also with first three abdominal segments free from palmate hairs. (See Plate 3, fig. 13.)

*A. Turkhudi.*

#### *The Habits of the Species.*

The habits of the species noted differ in many particulars from one another. This is especially the case in the selection of breeding-places, and the following groups can be distinguished:—

Group I. Open water breeders. Found in water with much aquatic vegetation—ponds, lakes, banks of rivers, swamps.

*A. barbirostris.*

*A. Sinensis*, sub-sp. *nigerrimus*.

Group II. Stream breeders. Found in swiftly running streams with grassy edges; also in irrigation ditches and nullahs.

*A. culicifacies.*

*A. Listonii.*

*A. Christophersi.*

Group III. Pool breeders:—

(a.) Those selecting clean pools with green alga, especially small pools left in river beds—

*A. Jamesii* (Liston).

*A. maculatus.*

*A. Theobaldi.*

*A. Lindesayi.*



(b.) Those found in small muddy pools—

*A. Rossii*.

*A. metaboles* (also breeds in pots and tins).

The adult insects of the first group are only occasionally caught in houses. They feed, however, upon human blood.

All other species, except *A. Lindesayi*, have been found by us in houses.

*Detailed Description of Species.*

*A. barbirostris*.

Ovum.

Upper surface very narrow.

Floats do not touch margin of upper surface.

Lower surface of ovum ornamented with polygonal markings.

Larva.

Antenna with large branched hair.

External pair of frontal hairs developed into cockades.

Palmate hairs on 2—7 abdominal segments.

Leaflets of palmate hairs lanceolate in shape and deeply serrated in outer half.

Head of larva without pigmented markings.

*A. Sinensis*, sub-sp. *nigerrimus* (see Report, No. VI).

Ovum.

Upper surface very narrow.

Floats do not touch margin of upper surface.

Lower surface of ovum ornamented with polygonal markings.

Larva.

Antenna with large branched hair.

External pair of frontal hairs developed into cockades.

Palmate hairs (?).

Leaflets of palmate hairs lanceolate with serrations in outer half.

Head of larva without pigmented markings.

The habits of both these species, *A. barbirostris* and *A. Sinensis*, sub-sp. *nigerrimus*, are very similar.

The larvæ are found in water with much aquatic vegetation—rivers, lakes, ponds, and swamps. They are only caught singly, but are generally widespread in their occurrence where large bodies of water are present.

The adults are not usually caught in houses. They seem to be attracted by lights, as on one or two occasions they were caught by us on a sheet illuminated by a lamp.

They are very common in Bengal. *A. barbirostris* was found by us scantily in the Central Provinces, whilst *A. Sinensis*, sub-sp. *nigerrimus*,

occurred in small numbers in the Punjab. Its frequency appears to depend upon the presence or absence of suitable sheets of water.

The female does not readily lay its eggs in captivity.

*A. fuliginosus* (Giles).

Ovum.

Upper surface moderately broad.

Floats touch margin of upper surface.

Floats long and narrow.

Fringe around upper surface only indicated by white border.

Lower surface not ornamented.

Larva.

Antenna without large branched hair.

External pair of frontal hairs branched (branches usually six in number).

Palmate hairs on 2—7 abdominal segments.\*

Leaflets of palmate hairs with very marked "shoulder" at origin of terminal filament. Terminal filament from  $\frac{1}{2}$ — $\frac{2}{3}$  length of basal portion. (Plate 4, fig. 15B.)

Head of larva with distinctive markings (see Plate 2, fig. 7).

This species was found in very large numbers in villages near Nagpur. It was breeding in considerable numbers, especially in small green pools in the course of a nullah. It was also caught by us in numbers in Calcutta Fort, which is surrounded by a moat. In other places visited by us it has only occurred in very small numbers.

In the Nagpur district it is the commonest species in December.

*A. Theobaldi* (Giles).

Ovum.

Unexamined.

Larva.

Similar to *A. Jamesii* (Liston) with the exception of the leaflets of the palmate hairs, in which the terminal filament is very short,  $\frac{1}{5}$ — $\frac{1}{6}$  length of basal portion. (Plate 3, fig. 8, and Plate 4, fig. 16.)

*A. maculatus* (Theobald) (see Reports to Malaria Committee, No. VI).

*A. culicifacies* (Giles).

Ovum.

Upper surface very narrow.

Floats do not touch margin of upper surface.

Lower surface not ornamented.

\* Rudimentary palmate hairs are frequently present upon other segments than those noted in the descriptions of larvæ. These are, however, so small as not to be mistaken for the functionally active hairs.

A short but distinct fringe is continued around margin of upper surface.

Larva.

Antenna without large branched hair.

Frontal hairs all unbranched.

Palmate hairs on 1—7 abdominal segment, and a pair of fairly developed ones upon the thorax.

Palmate hairs with terminal filament nearly as long as basal portion.

Head with markings (see Plate 2, fig. 10, and Plate 4, fig. 17).

Found in the irrigation ditches in Lahore; also in the running portion of the nullahs near Nagpur. It is the common stream mosquito in these places. The adults are found plentifully in native huts.

*A. Christophersi* (Theobald).

Ovum.

Upper surface very narrow.

Floats do not touch margin of upper surface.

Lower surface not ornamented.

A small fringe passes around margin of upper surface.

Larva.

Antenna without large branched hair.

Frontal hairs simple.

Palmate hairs on all segments, and very well developed pair on thorax.

The palmate hairs in this species are very large. The terminal filament is nearly as long as basal portion.

Found only in swiftly running streams with grassy edges in the Duars, at the foot of the Himalayas, in Northern Bengal. The common mosquito in the Duars.

*A. pulcherrimus* (Theobald).

Ovum.

Upper surface broad.

Floats touch margin of upper surface.

Fringe well developed around margin of upper surface. Striations are not present in that portion of the fringe lying over the floats.

Lower surface not ornamented.

Larva.

Antenna without large branched hair.

Outer pair of frontal hairs branched (six branches).

Palmate hairs on 2—7 abdominal segments.

Filament of palmate hair nearly as long as basal portion.

Head markings present.

Found only in Lahore, and sparingly there. Nature of breeding place unknown.

*A. metaboles* (Theobald).

Ovum.

Upper surface broad, except in central portion where encroached upon by floats.

Floats placed on margin of upper surface so that they touch, or nearly touch, one another in middle line. Floats short and almost globular.

Fringe not well developed.

Lower surface not ornamented.

Larva.

Antenna without large branched hair.

Frontal hairs unbranched.

Palmate hairs on 2—7 abdominal segments.

Leaflet of palmate hair with very short filament,  $\frac{1}{5}$ — $\frac{1}{8}$  length of basal portion.

Head usually with large black area covering the greater portion (see Plate 3, figs. 12, and Plate 4, fig. 19).

Found in Lahore in pots and tins, in which situation it was the only species present. In Nagpur, breeding in cattle-hoof marks, and other similar small pools by the side of nullah. A rare mosquito in Bengal and Punjab, but very common around Nagpur.

*A. Rossii* (Giles).

Ovum.

Upper surface broad.

Fringe very well developed and striated throughout whole length.

Floats scallop-shell shape and touch margin of anterior surface.

Lower surface not ornamented.

Larva.

Antenna without large branched hair.

Frontal hairs unbranched.

Palmate hairs 2—7 abdominal segments.

Terminal filament of palmate hair very long; often longer than basal portion. The "shoulder" at the origin of the filament is very slightly marked.

There are markings upon the head (see Plate 3, fig. 11, and Plate 4, fig. 18).

The commonest anopheles in all places visited by us, except Nagpur (in December). Breeds nearly always in small pools near houses. These pools are frequently foul, and nearly always muddy. The female lays her eggs very readily in captivity.



*A. Turkhudi* (Liston). (Plate 3, fig. 13, and Plate 4, fig. 20.)

*A. Turkhudi* is a very aberrant type, so far as the ovum and larva are concerned. Both the ovum and larva approach to the characters of the culex ovum and larva. The eggs were laid upon a floating object. When placed upon water they sank. They were laid in the heaped-up manner sometimes adopted by anopheles, especially *A. Rossii* and *A. Jamesii*. The chief characters of the ovum are :—

1. No separation of an upper surface as in all other anopheles ova. At the thicker end of the ovum there is an oval area about a quarter the length of the whole egg. This is glistening white and striated, and probably represents the upper surface of other anopheles ova.

2. There are no floats or any markings representing them.

3. There is a pale area at the thicker end of the egg, with a scalloped edge.

4. The ovum is otherwise without markings.

The larva is also culex-like in some of its characters, though undoubtedly much more nearly related to the anopheles type.

The full-grown larva is distinguished by the adoption of the slightly hanging attitude. The chief characters of the larva are :—

1. Two large additional frontal hairs are developed, which reach as far forward as the longest of the hairs, described in other larvæ.

2. The shape of the head varies from that of the ordinary anopheles larva.

3. The palmate hairs are only represented on two or three abdominal segments, namely, the 4th, 5th, and 6th. They are absent on the first three abdominal segments.

4. The palmate hairs are small and poorly developed. The leaflets are irregular and the terminal filament blunt.

This species must be looked upon as a form which in its egg and larval stages has lost many of the characteristics of anopheles eggs and larvæ, and has approached in these stages the characters of the eggs and larvæ of culex.

Nothing yet is known of its habits. It is found in houses, and feeds on human blood.

Taking into consideration all the characters known to us, it is evident that anopheles may be divided into some very natural groups, viz. :—

Group I. Large mosquitoes with black wings. Larvæ with branched hairs on antennæ, frontal tufts, and serrated lanceolate palmate leaflets. Ova of type 1.

The larvæ found in large bodies of water with much vegetation. Adults only occasionally frequenting houses.

The Indian species of this group are :—

*A. Sinensis* (Wied.).

*A. barbirostris* (Van der Wulp).

To this group also probably belongs the African species—

*A. paludis* (Theobald).

Group II. More or less dark mosquitoes. Larvæ with branched frontal hairs, but no branched hairs on antennæ or tufts. Ova of type 2. Larvæ found in clean pools in stream beds and elsewhere. Adults frequenting houses, but presumably also occurring wild.

The Indian species of this group are :—

*A. fuliginosus* (Giles).

*A. Theobaldi* (Giles).

*A. maculata* (Theobald).

Group III. The group of stream-loving anopheles, very small mosquitoes. Larvæ without branched hairs on antennæ and with unbranched frontal hairs, also with palmate hairs on every segment and the thorax.

Larvæ found in streams and ditches. Adults in houses.

The Indian species of this group are :—

*A. culicifacies* (Giles).

*A. Christophersi* (Theobald).

*A. Indicus* (Theobald).

*A. Listonii* (Giles).

To this group belongs the African species—

*A. funestus* (Giles).

Group IV. A group of domestic species. Light fawn-coloured mosquitoes. Larvæ without branched hairs on antennæ or branched frontal hairs. Also with palmate hairs only on two of abdominal segments. Larvæ found in pools by houses, also in springs, pots, and tins.

The Indian species are :—

*A. Rossii* (Giles).

*A. metaboles* (Theobald).

Group V. A very aberrant group containing at present only one species—

*A. Turkhudi* (Liston).

Group VI. *A. Lindesayi* is apparently entirely a hill species, and does not seem to be related to any other Indian anopheles. In the possession of a fairly well-developed branched hair on the antenna; it approaches the *A. Sinensis* group.

Appendix to paper on "The Classification of Indian Anopheles."

By J. W. W. STEPHENS and S. R. CHRISTOPHERS.

To the above we are able to add descriptions of the ova and larvæ of *A. Theobaldi* (Giles) and *A. Jamesii* (Theobald).

*A. Theobaldi* (Giles).

*Ovum*.—As the females of this species have only been very occasionally caught by us in houses, we have not been able to describe the ovum as deposited by the insect. Fully developed ova removed from a bred specimen showed, however, that the ovum resembled that of *A. Jamesii*. The floats were rather short and situated far forwards as in *A. Stephensii*. The fringe is fairly developed, but does not pass over the floats.

*Larva*.—Antenna without large branched lateral hair.

Frontal hairs unbranched. (Plate 3, fig. 8.)

Palmate hairs on 2—7 segments

Leaflets of palmate hairs have very short filaments. There are marked notches at the ending of the leaflet in the filament. (Plate 4, fig. 21.)

The larvæ of this species frequent especially sluggish streams with much growth of alga. They were found by us in Nagpur in association with *A. fuliginosus*, *A. barbirostris*, and *A. Listonii*.

The adults are only rarely caught in houses in our experience in Nagpur. The species is a very good carrier of the malignant tertian parasite when artificially fed.

*A. Jamesii* (Theobald).

*Ovum*.—(Plate 1, fig. 6).

The upper surface is rather narrow.

The floats are rather short and oval, and are placed far forwards as in the ovum of *A. Stephensii* though less markedly so.

The fringe is fairly developed, but is not continued over the floats.

*Larva*.—Antenna without large branched lateral hair.

Frontal hairs are peculiar and show a condition intermediate between the branched hairs of *A. barbirostris*, *A. fuliginosus*, and the unbranched hairs of *A. Rossii* and other species. (Plate 3, fig. 14, Plate 4, fig. 15.)

Palmate hairs on 2—7 segments.

Leaflets of palmate hairs have very short filaments. The notching at the termination of the leaflet is not so marked as in *A. Theobaldi*. (Plate 4, fig. 22.)

The larvæ of *A. Jamesii* (Theobald) have only been found by us in one situation, namely, a chain of small muddy puddles connected by trickling water.

The adults occurred in huts close at hand.

*A. fuliginosus* occurred in thousands in the same huts, breeding freely in a weedy lake. The larvæ of *A. Jamesii* (Theobald), however, could not be detected in the lake.

The three species *A. fuliginosus* (Giles), *A. Jamesii* (Theobald), *A. Theobaldi* (Giles) are in the adult condition very similar. Liston\* states that many forms intermediate between *A. Theobaldi* and *A. fuliginosus* occur and thinks these two forms may be but variations of one species. The fact that the frontal hairs of *A. Theobaldi* are simple, while those of *A. fuliginosus* are markedly branched, shows that this is not the case.

The differences between adult specimens of *A. fuliginosus* (Giles) and *A. Jamesii* (Theobald) are indeed so slight that one might consider them very closely related. The characters of the ovum and larva show, however, that *A. Jamesii* (Theobald) is more closely related to the group to which *A. Theobaldi* (Giles) belongs than to that of *A. fuliginosus*.

A group in which the larvæ are characterised by very short filaments of the palmate hairs would contain the following species—

<i>A. metaboles</i> (Theobald).	<i>A. Theobaldi</i> (Giles).
<i>A. maculatus</i> (Theobald).	<i>A. Jamesii</i> (Theobald).

It is also a characteristic of all these species that they have "speckled" legs.

With regard to the large branched hair on the antenna of *A. barbirostris* (Van de Wulp), and *A. nigerrimus* (Giles), we have noticed that a similar structure is very largely developed in some of the eulicidæ larvæ. We have been able to separate two forms of larvæ in this group—that of *A. barbirostris*, which is distinguished by the external frontal hairs being closely tufted so as to resemble a shaving brush, and another form which we believe to be the larva of *A. nigerrimus*, in which the external frontal hairs are loosely branched (see Plate 2, fig. 6A). We have, however, not yet succeeded in hatching out adults from this latter form of larva.

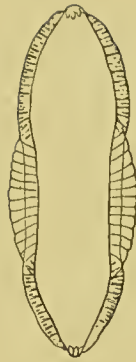
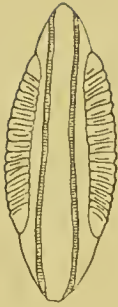
J. W. W. STEPHENS.  
S. R. CHRISTOPHERS.

\* Communication read at the Malaria Convention at Nagpur, Dec. 1901.

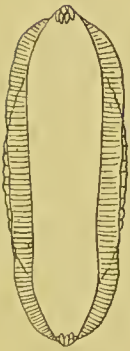




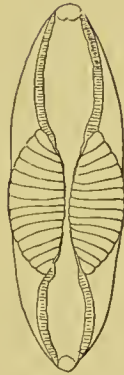
1.



2.



3.



4.



5.



6.



1. *A. culicifacies*,  
4. *A. Stephensi*.

2. *A. pulcherrimus*,  
5. *A. Turkhudi*.

3. *A. Rossii*,  
6. *A. Jamesii*.

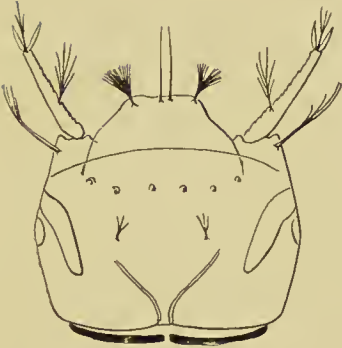




Pl. 2, 6A.



Pl. 2, 6B.



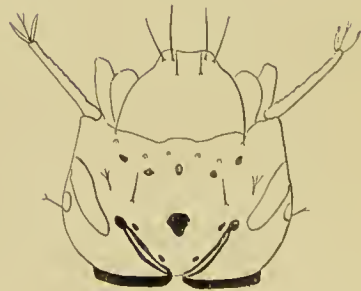
Pl. 2, fig. 6.



Pl. 2, fig. 7.



Pl. 2, fig. 9.



Pl. 2, fig. 10.

- |                             |  |                              |
|-----------------------------|--|------------------------------|
| 6. <i>A. barbirostris</i> . | 6A. <i>A. Sinensis</i> , sub-sp. <i>nigerrimus</i> . | 6B. <i>A. barbirostris</i> . |
| 7. <i>A. fuliginosus</i> .  | 9. <i>A. pulcherrimus</i> .                          | 10. <i>A. culicifacies</i> . |







Pl. 3, fig. 8.



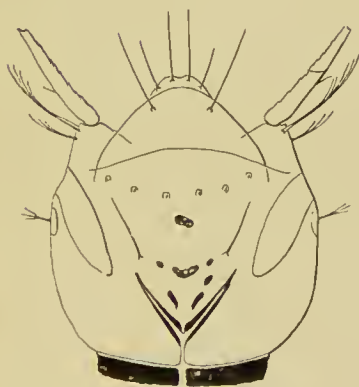
Pl. 3, fig. 10A.



Pl. 3, fig. 11.



Pl. 3, fig. 12.



Pl. 3, fig. 13.



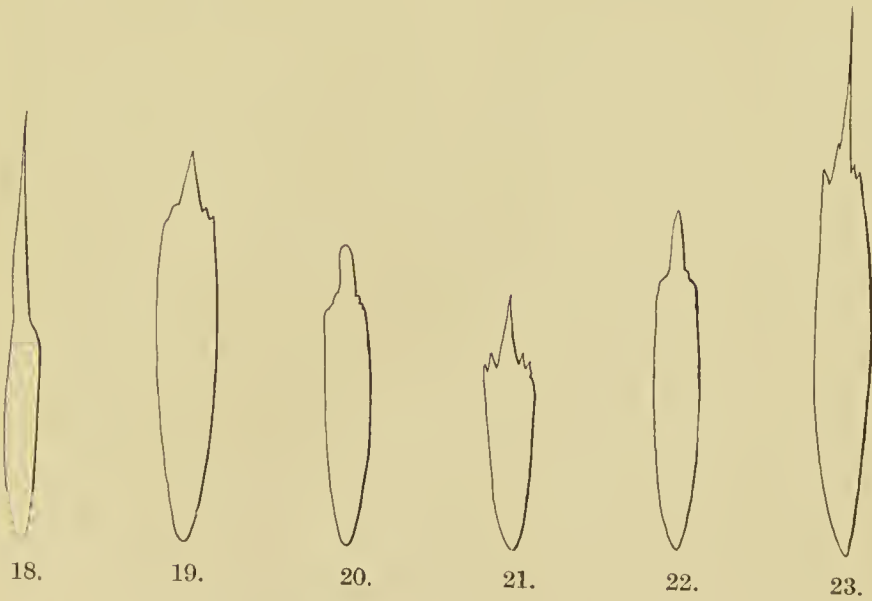
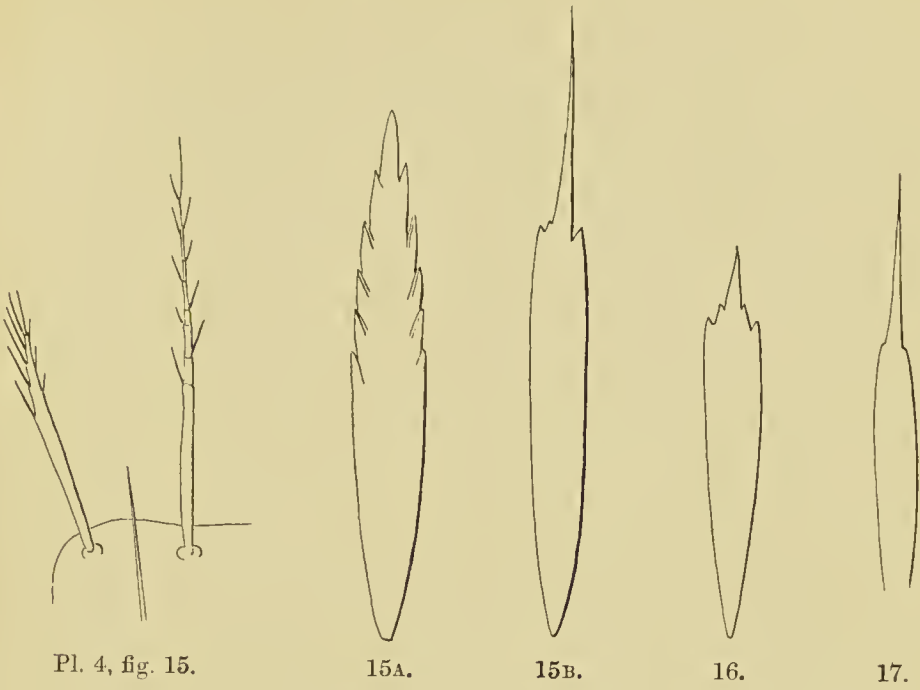
Pl. 3, fig. 14.

8. *A. Theobaldi* (Giles).  
12. *A. Stephensi*.

10A. *A. Listonii*.  
13. *A. Turkhudi*.

11. *A. Rossii*.  
14. *A. Jamesii* (Theobald).





15. *A. Jamesii* (Theobald).  
 15A. *A. barbirostris*.  
 15B. *A. fuliginosus*.  
 16. *A. Theobaldi*.  
 17. *A. culicifacies*.  
 18. *A. Rossii*.

19. *A. Stephensi*.  
 20. *A. Turkhudi*.  
 21. *A. Theobaldi* (Giles).  
 22. *A. Jamesii* (Theobald).  
 23. *A. Listoni*.

11 . 01 . 17 . 18 . 19 . 20 . 21 . 22 . 23 . 24 . 25 . 26 . 27 . 28 . 29 . 30 . 31 .

11 . 01 . 17 . 18 . 19 . 20 . 21 . 22 . 23 . 24 . 25 . 26 . 27 . 28 . 29 . 30 . 31 .

11 . 01 . 17 . 18 . 19 . 20 . 21 . 22 . 23 . 24 . 25 . 26 . 27 . 28 . 29 . 30 . 31 .



"The Relation of Species of Anopheles to Malarial Endemicity."

By J. W. W. STEPHENS, M.D., Cantab., and S. R. CHRISTOPHERS, M.B. Vict. Received February 6, 1902.

In a previous report we have shown that some marked variations in the malarial endemicity of Bengal encountered by us were possibly to be explained by a difference in the prevalent species of anopheles.

The conditions encountered there by us were briefly as follows:—

1. In parts of Calcutta *A. Rossii* existed in myriads in the native houses, but, notwithstanding this, there was no malarial infection of the native children nor sporozoites in the anopheles.

2. In the districts around Calcutta, and in several districts in Northern Bengal, *A. Rossii* was the common anopheles, though *A. Jamesii* and *A. Nigerrimus* were also generally to be found. The endemic index in many of these places was 0 per cent., in others it was higher, reaching in one case 16 per cent.

3. From the Mainaguri district, through the Bengal Duars, the endemic index was extraordinarily high, varying from 40 per cent. to 75 per cent. Throughout this district a small anopheles (*A. Christophersi*), resembling the African species *A. funestus*, was common, and in this we readily found sporozoites.

To elucidate this relationship of species to endemicity, there remained two methods at our disposal.

The first was the comparison of the infection of *A. Rossii* and a species that carried sporozoites under identical natural conditions.

The second was the experimental one of feeding on suitable cases at the same time *A. Rossii* and a known carrier.

We have confined our attention chiefly to *A. Rossii*, yet the same problem arises in connection with other species, and a comparison of the relative infection of different species under the same conditions requires investigation.

In Mian Mir (Punjab) we have lately had an opportunity of carrying out the first method of investigation.

Two species of anopheles were common in most of the bazaars, *A. Rossii* and *A. culicifacies*, a small anopheles belonging to the *A. funestus* group (see paper on "The Classification of Indian Anopheles"), and resembling closely *A. Christophersi*. *A. pulcherrimus*, *A. Stephensii*, and *A. Jamesii* occurred in such small numbers in Mian Mir that neither of these could well be the active agent in the transmission of malaria.

In the different bazaars of Mian Mir, infection in the children varied from 20 to 52 per cent.

A bazaar was chosen in which both *A. Rossii* and *A. culicifacies* were readily caught. An examination of the children showed that the spleen rate was 80 per cent. and the parasite rate 52 per cent.

We therefore knew that malaria was being carried by one or both of the species of anopheles.

Specimens of *A. Rossii* and *A. culicifacies* were caught at the same time in the same houses, three in number, in the midst of the bazaar.

In every situation a certain proportion of *A. culicifacies* carried sporozoits, whilst *A. Rossii* was uniformly negative.

The results of our dissections for sporozoits, extending over a month at the height of the fever season (October), were—

	Number dissected.	Number with sporozoits.*	Percentage with sporozoits.
<i>A. Rossii</i> .. .. .	464	0	Per cent. 0
<i>A. culicifacies</i> .. ..	252	11	4·3

so that at the height of the fever season *A. culicifacies* was the chief if not the only species concerned in the transmission of malaria.

It still remained to be seen whether these results would be confirmed by the experimental method. We were unable to continue our work in the Punjab on account of the onset of the cold season. Our feeding experiments were therefore undertaken in Nagpur, in the Central Provinces. Both anopheles caught in the villages† and those bred from larvæ were fed on cases of malignant tertian, simple tertian, and quartan infections. Two cases of malignant tertian were used, both having numerous flagellating forms.

A single case only of simple tertian was available, and this was not a severe infection. Flagellating bodies were, however, seen from time to time.

A case of quartan was also available. This case had a considerable infection of asexual forms, but sexual forms were only occasionally observed.

The results of the experiments were as follows:—

*A. Rossii* were unfortunately very rare, so that large numbers could not be used.

Mosquitoes caught in village.†

Fed since 10.12.01 on malignant tertian case.

Dissected 15.12.01.

\* In these dissections we did not examine the stomachs, wishing, in the first place, to determine merely the sporozoit rate in each species. In every case where sporozoits were found, films were made, fixed, and stained, but no differences in appearance or measurements were to be determined which could enable us to determine whether they were simple or malignant tertian or quartan.

† Results got with these were always confirmed with larval-bred anopheles.

*A. Jamesii*, a few young zygotes.

*A. Jamesii*, 3 young zygotes.

*A. Jamesii*, 3 young zygotes.

*A. Stephensi*,\* a single young zygote.

*A. Stephensi*, many young zygotes.

*A. culicifacies*, swarming with young zygotes.

Dissected 22.12.01.

*A. Stephensi*, swarming with medium-sized zygotes.

*A. Rossii*, a single medium-sized zygote.

Mosquitoes bred from larvæ.

Fed since 15.12.01 on malignant tertian case.

Dissected 23.12.01.

*A. Stephensi*, many rather small zygotes.

*A. culicifacies*, many young zygotes.

*A. culicifacies*, many rather young zygotes.

*A. culicifacies*, numerous rather young zygotes. Over 50 seen.

Dissected 25.12.01.

*A. Stephensi*, many medium-sized zygotes.

*A. Jamesii*, several young and one medium-sized zygote.

*A. culicifacies*, large number of medium-sized zygotes. 31 seen.

*A. culicifacies*, large number of small forms; also many medium-sized forms.

Mosquitoes bred from larvæ.

Fed since 19.12.01 on malignant tertian case.

Dissected 26.12.01.

*A. Rossii*, neg.

*A. Turkhudi*, 6 medium-sized zygotes.

Mosquitoes caught in village.

Fed since 18.12.01 on malignant tertian case.

Dissected 26.12.01.

*A. Stephensi*, 4 small zygotes, 12 medium-sized zygotes.

*A. Jamesii*, extremely numerous zygotes, 66 medium, 10 small.

*A. culicifacies*, 4 rather large zygotes.

Mosquitoes bred from larvæ.

Fed since 19.12.01. on malignant tertian case.

Dissected 27.12.01.

\* *A. Stephensi* (Liston). Syn. *A. metaboles* (Theobald).

*A. Turkhudi*, a few medium-sized zygotes.

*A. Turkhudi*, a small zygote.

*A. Stephensi*, large numbers of zygotes, 10 or more in some fields.

Mosquitoes caught in village.

Fed since 14.12.01 on malignant tertian case.

Dissected 28.12.01.

*A. Stephensi*, 24 small zygotes, 266 medium-sized, 42 large zygotes.

*A. Rossii*, a few young zygotes.

Mosquitoes bred from larvæ.

Fed since 22.12.01 on malignant tertian case.

Dissected 28.12.01.

*A. Rossii*, 1 small, 6 medium-sized zygotes.

*A. Rossii*, 7 small, 9 medium-sized zygotes.

*A. Rossii*, 1 small zygote. Blood not entirely left stomach.

*A. Rossii*, 12 small, 22 medium-sized zygotes.

Mosquitoes caught in village.

Fed since 21.12.01 on malignant tertian case.

Dissected 30.12.01.

*A. Stephensi*, 1 medium-sized, 12 large zygotes.

*A. Stephensi*, over 50 large forms.

*A. Jamesii*, 7 small forms.

Mosquitoes bred from larvæ.

Fed since 25.12.01 on malignant tertian case.

Dissected 6.1.02.

*A. barbirostris*, negative.

*A. barbirostris*, negative.

*A. Stephensi*, very numerous, nearly fully developed zygotes.

Mosquitoes bred from larvæ.

Fed from 15.12.01 on malignant tertian case.

Dissected 18.12.01.

*A. Theobaldi*, many young zygotes.

Mosquitoes bred from larvæ.

Fed for 6 days on simple tertian case.

*A. culicifacies*, a few medium-sized zygotes with simple tertian pigment.

*A. culicifacies*, negative.



As a result of these experiments, we can say that so far as the development of zygotes is concerned, the following species of anopheles are carriers of the malignant tertian parasite :—

<i>A. culicifacies.</i>	<i>A. Theobaldi.</i>
<i>A. Stephensi.</i>	<i>A. Rossii.</i>
<i>A. Jamesii.</i>	<i>A. Turkhudi.</i>

The only species which has seemed not to carry zygotes is—

*A. barbirostris.*

As only two specimens, however, have been fed, this result may not be confirmed by further experiments.

Although each of the above species is capable of carrying the zygotes of malignant tertian, yet some difference in the numbers of zygotes found was noted. With one exception in the above experiments, *A. Jamesii* showed only a small number of zygotes, although *A. Stephensi* and *A. culicifacies* in the same batch frequently showed very large numbers.

*A. Rossii* and *A. Turkhudi*, both undoubtedly carry zygotes. *A. culicifacies* and *A. Stephensi* have always in our experiments shown, however, larger numbers.

Our experiments with simple tertian cases were generally negative. The zygotes of this parasite were, however, seen in *A. culicifacies*.

Experiments with the quartan case have so far been entirely negative.

Further experiments both by dissection of anopheles under natural conditions and by experimental infection are necessary before we can estimate the part played by species in the distribution of malaria. The distribution of quartan and tertian parasites we have shown is peculiar in India. Thus in the Bengal Duars the parasite was almost exclusively quartan, whilst in Mian Mir (Punjab) quartans were not found by us, by far the commonest parasite being simple tertian. What part species may play in this peculiar distribution is not yet evident.

[We are much indebted to Major Buchanan, I.M.S., for the facilities afforded us in the Central Jail, Nagpur.]



"The Relation of Species of Anopheles to Malarial Endemicity.—Further Report." By J. W. W. STEPHENS, M.D. Cantab., and S. R. CHRISTOPHERS, M.B. Vict. Received April 25, 1902.

In two previous reports we have considered this question. In the first we were led to suggest that species was a determining factor in endemicity from our observations in Bengal.

We found that in the outskirts of Calcutta there were innumerable anopheles, yet the endemic index was nil. The species found in Calcutta was in the main *A. Rossii*. *A. fuliginosus*, *A. sinensis*, sub. sp. *mgerrimus*, and *A. Stephensi* also occurred.

At Ranaghat, 50 miles from Calcutta, the infection was 14 per cent. At Jalpaiguri, still in the plains, the infection was 16 per cent. At Mainaguri, also in the plains, but on the borders of the Duars, the infection was 25 per cent.

In the Duars, *i.e.*, at the foot of the Himalayas at an elevation of about 800 feet, there was a sudden marked rise in the endemic index, reaching at Nagrakata 72 per cent.

The fact that in the Duars a new anopheles fauna was encountered, the common mosquito being *A. Christophersi*, led us to think that this factor might possibly be the determining cause of the high endemic index.

*A. Christophersi* is evidently closely allied to the African species *A. funestus*, and as we found later belongs to a group, members of which we have met in another district with an equally high or even higher endemic index.

We showed that dissections of two species, *A. Rossii* and *A. culicifacies*, caught under the same conditions from the same houses gave a very different result. To this point we shall return later in this report.

We showed that in spite of these results, every species but *A. barbirostris* carried malignant tertian when fed experimentally on suitable cases. Since then we have found well-developed zygotes in *A. barbirostris* fed on malignant tertian. We found also that the benign tertian parasite also developed in many species of anopheles.

There was, however, noticeable a difference in the number of zygotes which usually developed in certain species, and the number in certain other species fed at the same time on the same case.

The species which appeared to be most active were:—

*A. culicifacies*, *A. Stephensi*, *A. Theobaldi*.

Those which seemed less susceptible were:—

*A. Rossii*, *A. barbirostris*, *A. fuliginosus*.

Lately we have fed anopheles on quartan cases. We may note incidentally, that in other series of feeding experiments we have not found it necessary that the anopheles should be fertilised. Unfertilised anopheles were found by us to convey parasites readily, at least as far as the stage of large zygotes.

The anopheles bred from larvæ were fed every night in rotation on four cases of regular quartan. Four different cases were used, because in only one case on one occasion could a flagellating body be found. The conditions were thus presumably unfavourable, and the four cases were used in the hope of producing infection of the mosquito.

Table I.—Results of feeding Various Species of Anopheles on Quartan Cases.

Date of feeding.	Species of anopheles.	Dissection of stomach.	Dissection of glands.
17th—28th Ditto Ditto	<i>A. fuliginosus</i> Ditto Ditto	Nil Nil Two small zygotes	Nil. Nil. Nil.
13th—28th Ditto Ditto Ditto Ditto Ditto Ditto Ditto Ditto	<i>A. culicifacies</i> Ditto Ditto Ditto <i>A. Theobaldi</i> Ditto <i>A. culicifacies</i> <i>A. Theobaldi</i> Ditto	— Large zygote Nil Nil Nil Nil Small zygote Nil Small zygote	Sporozoits scanty. Ditto. Nil. Nil. — Nil. Nil. Nil. Nil.
17th—28th Ditto Ditto	<i>A. fuliginosus</i> Ditto Ditto	Nil Large zygote Nil	Nil. Nil. Nil.
16th—28th Ditto Ditto Ditto Ditto Ditto Ditto Ditto	<i>A. Rossii</i> <i>A. culicifacies</i> <i>A. Rossii</i> <i>A. Stephensi</i> <i>A. Rossii</i> <i>A. Stephensi</i> Ditto Ditto	Small zygote Large zygote — Two small zygotes Small zygote Nil Nil Nil	Nil. Nil. Nil. Nil. Nil. Nil. Nil. Nil.

The results (Table I) show that zygotes have developed in four species besides *A. culicifacies*, while in the latter only have sporozoits developed. We would not lay too much stress on this point, as the conditions were unfavourable, but we would point out that *A. culici-*

*facies* carries quartan sporozoits in nature, while *A. Rossii*, as we shall see later, does not.

Finally we have been able to again find a village of high endemicity where a comparative dissection of the two species occurring in the huts together was possible. Ennur,\* a fishing village, 10 miles from Madras, is intensely malarious. We found on examination that the spleen rate was 95 per cent., and the endemic index 54. At first sight it appeared as if *A. Rossii* was the only species present in the huts, for they swarmed in the dirty thatch. Careful search, however, detected also *A. culicifacies*, but while after nearly a week's search only 69 *A. culicifacies* were collected, *A. Rossii* could be caught in hundreds. Here an observer with insufficient experience might reasonably have attributed the high endemic rate of Ennur to the abundance of *A. Rossii*. Dissection here, as in Mian Mir, has conclusively proved that under identical conditions *A. Rossii* is not carrying malaria while *A. culicifacies* is.

It may be noted that in Ennur the infection was almost exclusively quartan, while in Mian Mir it was benign tertian.

The results of our dissections in Mian Mir and Ennur are as follows :—

#### 1. Mian Mir.

	Anopheles dissected.	Number with sporozoits.	Percentage, sporozoits.
<i>A. culicifacies</i> .....	259	12	4·6 per cent.
<i>A. Rossii</i> .....	496	0	0 „

#### 2. Ennur.

	Anopheles dissected.	Number with sporozoits.	Percentage, sporozoits.
<i>A. culicifacies</i> .....	69	6	8·6 per cent.
<i>A. Rossii</i> .....	364	0	0 „

We consider then that in spite of the results of feeding experiments *A. Rossii* does not in nature generally carry sporozoits, and is negligible as a carrier of malaria.

A possible explanation is that *A. Rossii* does not live a sufficient time to allow of the formation of sporozoits. Our observations, however, do not favour the view that *A. Rossii* is a short-lived mosquito. The explanation of the difference between this species and *A. culicifacies* is obscure. We may mention that in Ennur zygotes were found in the stomach of one of a small number (18) of *A. Rossii* dissected (observation of Captain S. P. James, I.M.S.).

We have already alluded in previous reports to the general reasons

\* We are much indebted to Captain James, I.M.S., for bringing this village to our notice.



which have led us to suppose that other species are also not actively concerned in the transmission of malaria. Thus, for instance, *A. fuliginosus*, *A. sinensis*, and *A. barbirostris* do not seem to be associated with a high rate of infection. The group of small dark mosquitoes with unbanded legs, *A. Christophersi*, *A. Jeyporensis*, *A. Listonii*, *A. culicifacies*, *A. indica*, appear to be eminently the carriers of malaria, and are associated with areas of high endemic index. Two of these, *A. culicifacies* and *A. Christophersi*, have been shown by us to be actively concerned in the spread of malaria in India.

Further observations on the sporozoite rate of associated species are needed in this connection, also for more accurate and detailed knowledge of the distribution of the different species of anophelids and malaria. Whether virulence of infection may depend on such a factor is at present only a matter of conjecture.

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“An Investigation into the Factors which determine Malarial Endemicity.” By J. W. W. STEPHENS, M.D. Cantab., and S. R. CHRISTOPHERS, M.B. Vict. Received April 25, 1902.

Information concerning endemic malaria in different countries in the world is meagre. Statements that certain countries are highly malarious while others are comparatively free from malaria, do not allow of accurate comparison between the two. It is only since Koch used the percentage of infected children to obtain a definite figure relative to the prevalence of malaria, that it became possible to compare accurately the endemicity of one district with another. Thus Koch showed that the endemicity of villages in the Dutch East Indies varied from 0 per cent. to 100 per cent.

We have shown that in Sierra Leone, the Gold Coast, and in Lagos a high rate prevails, most frequently approaching 100 per cent. Annett and Dutton also found a similar but somewhat lower rate in most villages in Nigeria. Ziemann, in the Cameroons, also finds a high endemic rate among the children.

Cropper recently,\* judging by the spleen rate, shows that areas of high endemicity occur in certain districts in Palestine. He also notes the occurrence of blackwater fever in these districts.

In India we have found areas of high and others of extremely low endemic index. Thus, in the swampy plains of Bengal, over hundreds of miles in extent, the endemic index was uniformly low. Within 30 miles this rate increased until an area, the Bengal Duars and Darjeeling

\* ‘Journal of Hygiene,’ vol. 2, No. 1, p. 47.

Terai, extending for some hundreds of miles, and having an endemic index uniformly high (40—70), was entered.

A similar area of high endemicity has recently been investigated by us in the hill districts of the Jeypore Agency, Madras, where also a striking contrast occurred between the high endemic index of the hill regions and the low index of the adjacent plain districts. It may be mentioned incidentally that in both these regions of intense malaria, blackwater fever is a well-known disease.

With a view to the possible elucidation of such diversities in endemicity as these observations had revealed to us, we determined to examine systematically the causes which influence endemic malaria.

For this purpose we have found that small native villages afford suitable opportunities for observation. The conditions can be determined with some degree of accuracy, and it is possible to select villages where extreme dissimilarities in regard to local conditions are present.

While bearing in mind the possible influence on endemicity of unknown epidemiological factors, we endeavoured in the first place to estimate how far known factors, especially the presence of anopheles, or the extent and proximity of breeding grounds, would account for variations in the parasite rate.

#### *The presence of Breeding Grounds.*

(a.) *Extent of Breeding Ground.*—In many villages, especially those where wet irrigation exists, the facilities for breeding are practically unlimited. In other cases a half-dried river bed affords abundance of pools with much weedy growth. On the other hand, the breeding grounds may be scanty and insignificant.

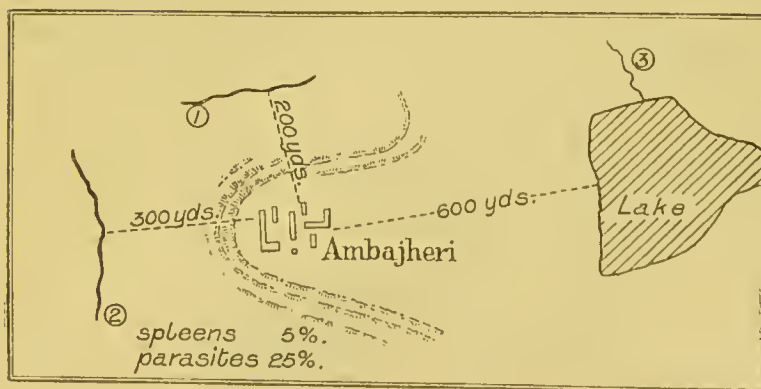
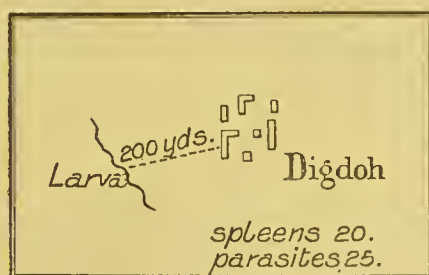
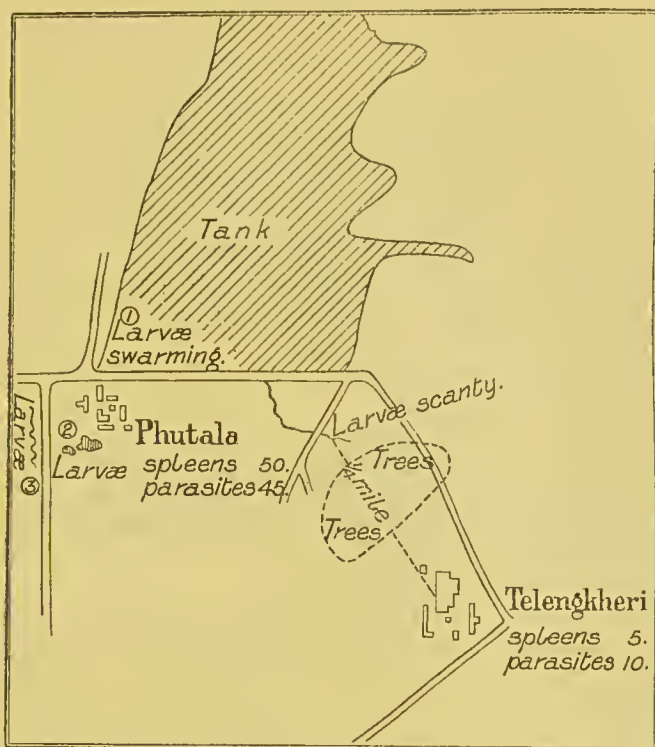
(b.) *Distance of Breeding Grounds.*—In swampy districts the village is usually situated in the midst of much shallow water, and breeding places are often within a few yards of the houses. In other villages the breeding grounds are often 200 or 400 yards away. Villages may occasionally be found in which breeding places are not present within as much as half or even one mile.

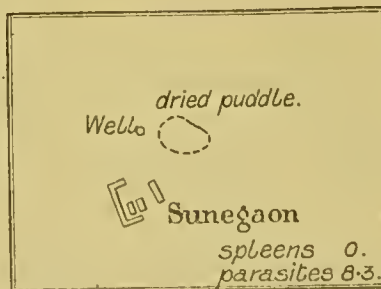
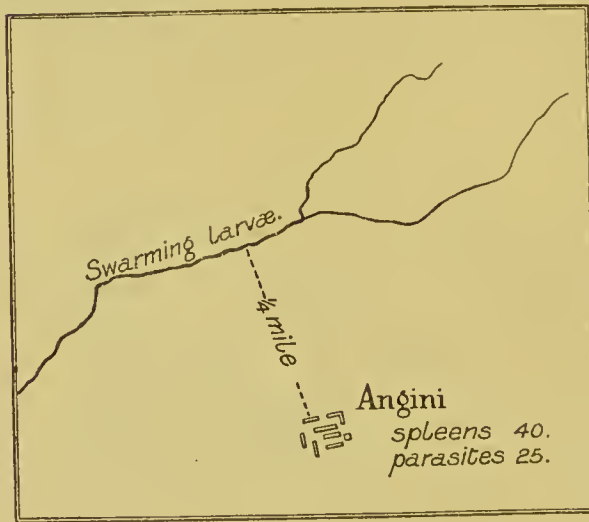
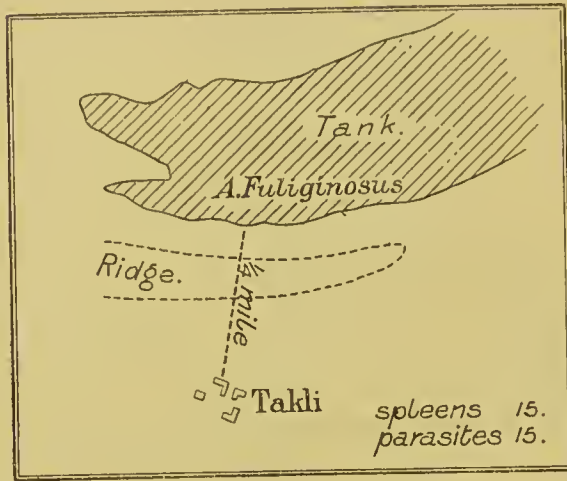
The influence of breeding grounds is shown very clearly in the observations made by us in Nagpur, Central Provinces. Here over a great extent of country, for a considerable part of the year, the breeding places are closely confined to small half-dried-up watercourses.

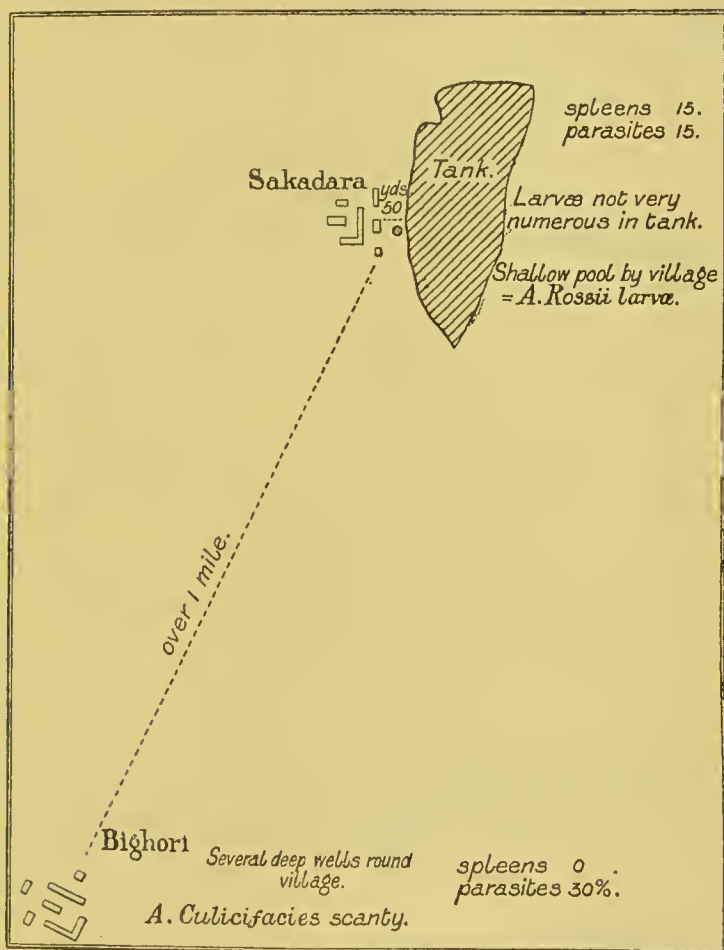
The contrast between “dry” villages with breeding places not nearer than half a mile, and “wet” villages where there were breeding places immediately at hand, is in this district a marked one.

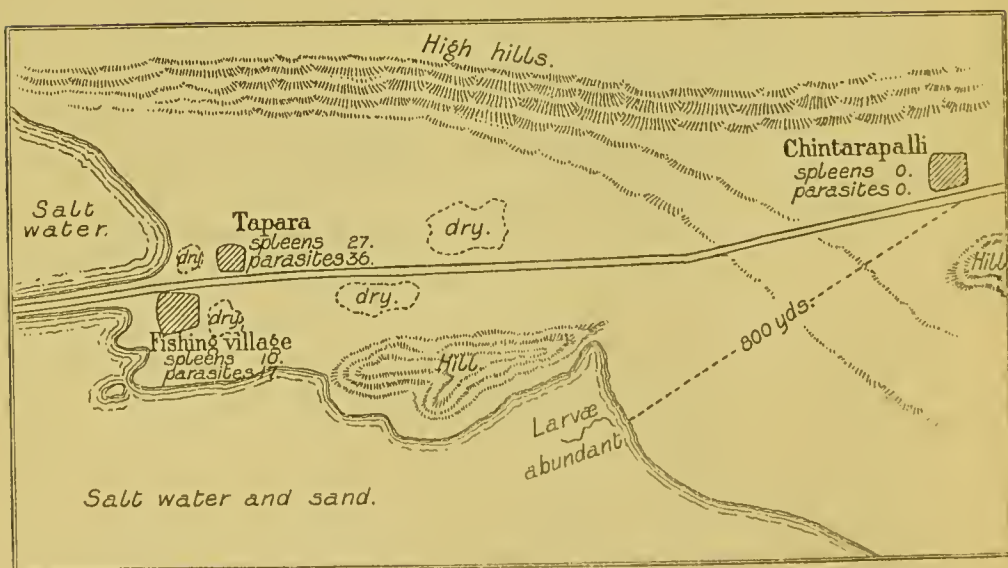
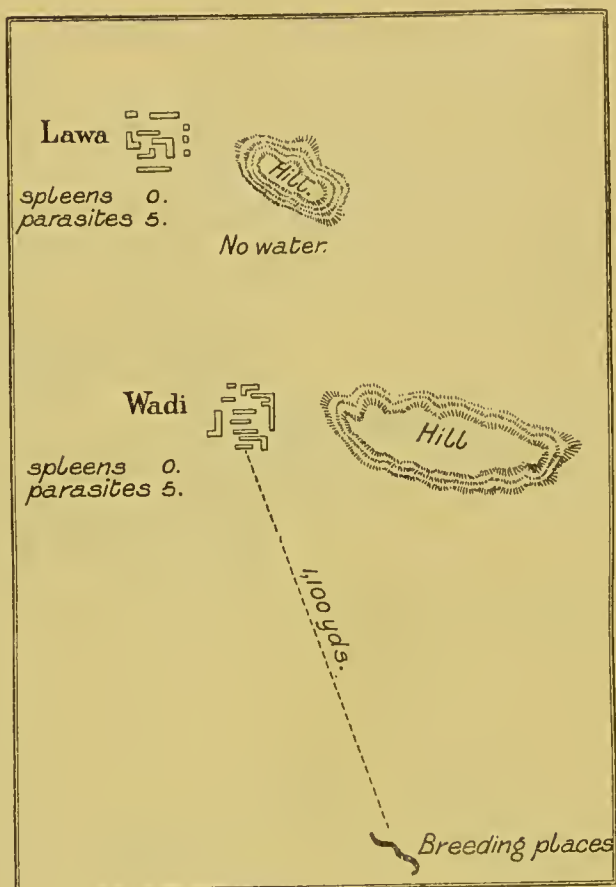
The accompanying plans show accurately the distances of the nearest water, a point which could always be accurately determined, owing to the physical character of the country.

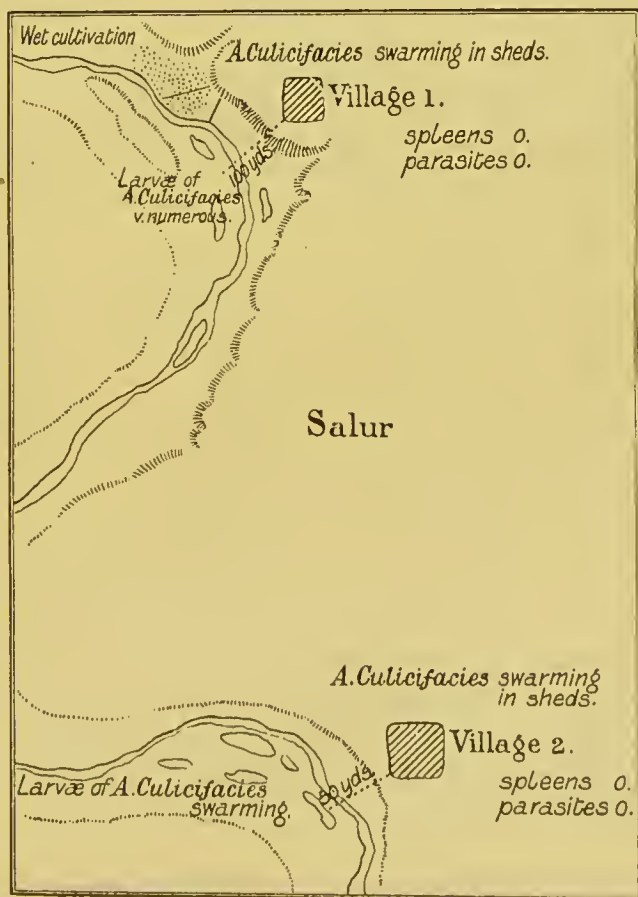










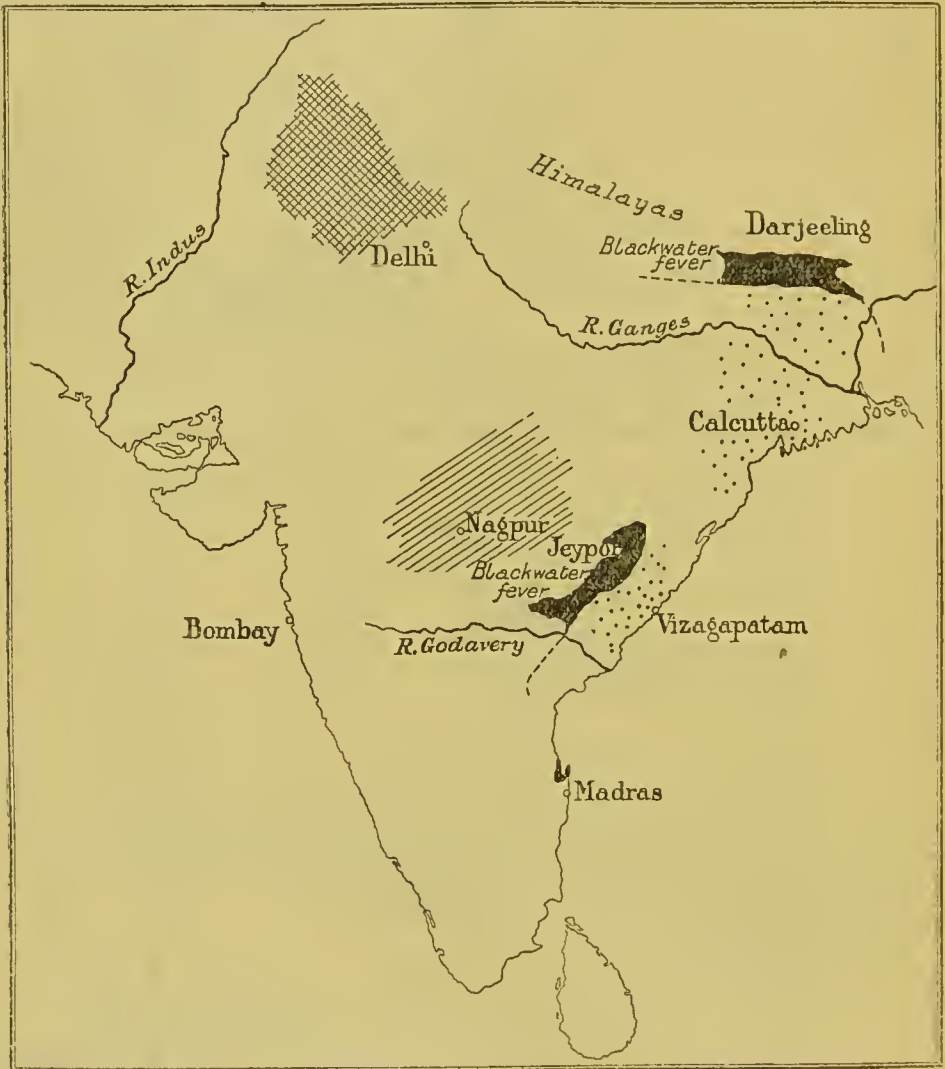




The variations in the endemic index under conditions varying with regard to breeding places are as seen in the table (Table I).

It is evident that in this district there is a close relationship between the distance of breeding grounds and

- (1.) The number of anopheles in the houses.
- (2.) The endemic index.



Villages may be considered in three classes.

1. Those with extensive breeding grounds close at hand, plentiful anopheles in the houses, and a high endemic index and spleen rate. In the above series, Phutala exemplifies these conditions, and was quite exceptional in the Nagpur district, the endemic index being 50 (January).

2. Those with extensive breeding grounds up to quarter of a mile

Table I.—To show Relation between the Distance and Extent of Breeding Places, the Number of Anopheles in the Houses, and the Amount of Malaria.

Villages.	Parasite rate.	Spleen rate.	Species of anopheles found.	Distance and extent of breeding grounds.
Phutala .....	45	50	<i>A. culicifacies</i> } Very abundant <i>A. fuliginosus</i> }	Extensive. Less than 50 yds.
Sakadara.....	15	15	.. ..	<i>A. Rossii</i> } Not very abundant. Less <i>A. fuliginosus</i> } than 50 yds.
Digdoh .....	20	25	<i>A. culicifacies</i> . Very abundant [ <i>A. fuliginosus</i> ]	100 yds. distant.
Leendra .....	20	0	<i>A. culicifacies</i> } Abundant <i>A. fuliginosus</i> }	200 yds. distant.
Ambajheri .....	25	5	<i>A. fuliginosus</i> . Abundant [ <i>A. culicifacies</i> ]	200 yds. distant.
Angini .....	25	40	<i>A. culicifacies</i> } Very abundant <i>A. Stephensi</i> } <i>A. fuliginosus</i> }	$\frac{1}{4}$ mile. Very extensive.
Babulkera .....	—	20	<i>A. culicifacies</i> } Very abundant <i>A. Stephensi</i> } <i>A. fuliginosus</i> }	$\frac{1}{4}$ mile. Extensive.
Takli .....	15	15	<i>A. Listonii</i> } Scanty <i>A. culicifacies</i> } <i>A. fuliginosus</i> }	$\frac{1}{4}$ mile. Extensive. Ridge intervening.

Type 1.

Villages.	Parasite rate.	Spleen rate.	Species of anopheles found.	Distance and extent of breeding grounds.
Type 1. { Telankeri ..... Kaamla .....	10	5	<i>A. fuliginosus</i> . Scanty	$\frac{1}{4}$ mile. Inextensive. Trees intervening.
	7	0	<i>A. culicifacies</i> } Scanty <i>A. Stephensi</i> }	$\frac{1}{4}$ mile. Inextensive.
Type 2. { Sunegaon ..... Bighori .....	8	0	<i>A. Stephensi</i> . Scanty	No breeding places within $\frac{1}{2}$ mile. Dried-up buffalo wallow within 50 yds.
	30	0	<i>A. culicifacies</i> . Scanty	No breeding place within $\frac{3}{4}$ mile.
Type 3. { Lawa ..... Wadi .....	5	0	Nil	Breeding places over $\frac{1}{2}$ mile.
	5	0	Nil	Breeding places over $\frac{1}{2}$ mile.

away. These also generally have numerous anopheles in the houses, and a moderately high endemic index. The endemic index in the Nagpur district under such conditions was on an average 25 per cent. (January).

3. Those with breeding places over half a mile distant. These showed:—

a. Anopheles not to be detected and a much reduced endemic index (5 per cent., or in some cases 0 per cent.).

b. A variable number of anopheles in the houses, and often a considerable amount of malaria.

We shall consider further this latter class of villages, as they are of considerable interest, and as we have already recorded in Africa conditions which seem exactly parallel. Though at first sight the presence of malaria in such villages is hard to account for, yet the explanation is, we believe in every case, a very simple one. In our second report (Gold Coast), we described the conditions found by us in a small isolated hut on the bed of a dried lagoon. We were able here to trace clearly the following cycle of events, which probably gives the clue to the apparent anomaly of malaria existing where there are no breeding grounds. When first visited, the hut had several shallow pits close at hand, and numerous anopheles were caught. Later the pools dried up, and there were then no breeding places anywhere in the neighbourhood. Anopheles were still caught, however, several weeks later. Later still they decreased in numbers, but the children still showed a high infection. In the accompanying table many examples of such a condition are shown.

*Presence (quantity) of Winged Anopheles.*

There are the following types of villages:—

Class A. Including—

1. Those with no anopheles and no malaria.
2. Those with anopheles more or less abundant, and a varying endemic rate.
3. Those with anopheles scanty or absent, and yet often with a fairly high endemic rate.

Class B. Villages with abundance of anopheles, and yet no malarial infection.

Examples of type (1), Class A, are Wadi, Lawa, and Chintarapalli (see maps, p. 28). It appears clear that an absence of breeding grounds within half a mile continued throughout the year or for long periods (many months) is sufficient to ensure an absence of anopheles, and so little or no malaria.

Observations on villages of this type have indirectly thrown light on the question of the distance of flight of anopheles. In many

Table II.—Showing the continuation of Anopheles after Breeding Places have Dried up and Presence of "Residual" Malaria.

	Parasite rate.	Spleen rate.	Anopheles in houses.	Breeding places at time of observation.	Signs of extensive breeding grounds some time previously.
District 1. Accra, Gold Coast.	—	—	Abundant.....	Yes	Yes.
	70	—	Scanty.....	Scanty	Yes.
	31	—	No.....	No	No.
	63	—	Scanty.....	No	Yes.
	—	—	Very scanty.....	No	Yes.
	69	—	—	No	—
	61	—	Abundant.....	Yes	Yes.
	—	—	Abundant.....	Yes	—
	—	—	Abundant.....	No	—
	—	—	Abundant.....	No	—
District 2. Mian Mtr. Punjab.	52	80	Abundant <i>A. Rossii</i> and <i>A. culicifacies</i>	Yes	Yes.
	35	75	Abundant <i>A. Rossii</i> , scanty <i>A. culicifacies</i>	No	Yes.
	29	34	<i>A. Rossii</i> scanty, <i>A. culicifacies</i> not seen	No	Yes.
	—	—	Abundant <i>A. Rossii</i> , abundant <i>A. culicifacies</i>	Yes	—
	56	48	Very scanty <i>A. Rossii</i>	No	No.
	12	30	No anopheles	No	No.
	—	—	—	—	—
	—	—	—	—	—
	—	—	—	—	—
	—	—	—	—	—



	Parasite rate.	Spleen rate.	Anopheles in houses.	Breeding places at time of observation.	Signs of extensive breeding grounds some time previously.
District 2. Lahore. Punjab.	20	20	No anopheles found . . . . .	No	Doubtful, nearer than 600 yards.
	24	36	<i>A. Rossi</i> very scanty. . . . .	No	Yes.
	—	11	ditto . . . . .	No	
District 3. Vizagapatam.	0	0	No . . . . .	No	No.
	27	36	Scanty . . . . .	No	Yes.
	10	17	Scanty . . . . .	No	Yes.
	0	0	No . . . . .	No	No.
{ Syce lines, 54th Battery, November Cavalry lines— November. . . . . December. . . . . }					
{ Vizagapatam busy. Tapara . . . . . Fishing village. . . . . Chintarapalli . . . . . }					

statements on the flight of mosquitoes, it would appear that they apply to *eulex*. The question ought to be determined if possible, however, for the different species of anopheles, as considerable differences in the power of flight may well exist. In the villages in Nagpur the three common species are (December, January, and February) *A. culicifacies*, *A. Stephensi*, and *A. fuliginosus*. Where their breeding places are not removed over a quarter of a mile, all these species are found in abundance in houses. Much over a quarter of a mile, however, they do not occur; and at half a mile from extensive breeding places, houses contain few or none. At Mian Mir we failed after prolonged search to find any winged anopheles in huts 600 yards from breeding places. We may say with some certainty that *A. culicifacies*, *A. Stephensi*, and *A. fuliginosus* readily fly a quarter of a mile, but they do not in any numbers traverse half a mile.

The character of the land intervening undoubtedly has a great influence in this respect. This is especially noticeable in the case of villages with belts of trees between them and distant breeding grounds. The trees in this case form a protective screen.

In the second and third types of Class A, breeding grounds are either present, or there may be found signs that breeding grounds have more or less recently dried up, or the conditions are such that extensive breeding grounds must have existed during the rains. These two latter are exceedingly common conditions, and a clear realisation of the process concerned much simplifies observations on the relation of anopheles to malaria.

We have in these cases, so to speak, residual anopheles remaining behind a considerable time after their breeding places have disappeared, and only very gradually diminishing in numbers. Associated with this, we have a residual malarial infection, and this appears to persist still longer than the anopheles. Some degree of this condition is perhaps the commonest of all conditions found in villages in the dry season.

Class B.—Abundance of anopheles with no endemic malaria.

Nuttall, Cobbett, and Pigg showed, in 1900,\* that in the fen districts and elsewhere in England, *Anopheles maculipennis* were abundant, though presumably malaria is not now present.

Celli has also recorded areas where anopheles are abundant and imported fever cases frequent, yet there is no dissemination of malaria. In the outskirts of Calcutta we found abundance of anopheles (*A. Rossi*), yet no endemic malaria. Further we have in Salur, Madras, and a village near Vizagapatam two instances of this kind.

At Salur, *A. culicifacies* (which elsewhere has been found infected by us) occurs in profusion, the breeding grounds being close at hand and extensive, yet there is no splenic enlargement and no malarial

\* 'Journal of Hygiene,' vol. 1, No. 1.

infection. The possibility of infection from a neighbouring area of high infection is a constant one, yet there is no malaria there. Further observation of similar instances are necessary before any explanation can be satisfactory.

Summing up, these observations show that, except in certain peculiar instances, in any one district a direct relationship exists between the extent and proximity of breeding grounds, the number of anopheles, and the amount of malaria.

*Species of Anopheles.*—Nuttall has drawn attention to the importance of species in matters relating to the life-history of anopheles. Daniels has suggested the possibility of species being important.

Our reports on the natural history of Indian anopheles show that the habits of different species differ. Further, from a consideration of the ova, larvæ, and adult insects, we have been able to divide Indian anopheles into several natural and distinct groups.

In an accompanying report we have given the facts which led us to conclude that the species of anopheles was an important factor in determining the carrying of infection. Briefly they were of two kinds:—

Firstly, two species have been dissected by us in two different localities under identical conditions of capture, and of these *A. culicifacies* has in both instances carried sporozoits while *A. Rossii* has not. Experimentally we have shown both species will develop sporozoits, but this does not invalidate the result of our dissections in which a most undoubted difference in the sporozoit rate is shown in the two species.

Secondly, we find in regions of high endemicity that the carriers of infection belong to a group of mosquitoes which have common characteristics. They are small dark anopheles with unbanded legs. They include *A. culicifacies*, *A. Christophersi*, *A. Listonii*, *A. Indicus*, *A. Jeyporensis*. It is noteworthy that *A. Christophersi* found by us in the high endemic area of the Duars is closely allied to, but not identical with, *A. funestus*, the African species, which is the carrier in many parts of tropical Africa. It is closely allied also with *A. Jeyporensis* found by us in the high endemic area of the Jeypore agency.

We may also remark that since leaving the Duars we have not encountered *A. maculata*.\* This species does not appear to occur in the Bengal plains, in the N.W. Provinces, the Punjab, or the Central Provinces with its rich anopheles fauna. In the Jeypore hills, however, we again met with this species. At Sam Sing (3000 feet), Bengal Duars, this species was found alone and associated with an endemic index of 25.

The anopheles fauna of the Duars and the Jeypore hills, two regions of intense endemicity, is almost identical.

\* In Report VI, *A. metaboles* has throughout been erroneously printed for *A. maculatus*.



We can say that the abundance of anopheles has no relation to malaria when one species at least of anopheles, *A. Rossii*, only is concerned. The species of anopheles present indeed is, in all probability, a powerful determining cause of malarial endemicity.

### *Race and Social Status.*

Considerable differences in this respect were encountered by us in the various villages examined. Thus we find:—

1. Aboriginal tribes living in hill districts.
2. Plains people living in the hills.
3. People of high caste living under good conditions.
4. People of low caste living under squalid conditions.

Koch has attributed to the importation of non-immune immigrants into an immune district an increase of fever and a gradual subsidence of the fever rate as immunity is again acquired.

We, however, find intense malarial infection also among aboriginal tribes, where this factor is not in operation. Indeed, many of our highest endemic indices have been encountered in aboriginal villages. The presence of a high endemic index does not therefore depend upon an importation of strangers. (Table III.) The same condition of intense malaria also prevails in villages of plains people in the hills.

Children of low social status frequently showed a high malarial infection. This is no doubt partially due at any rate to their squalid dwellings being suitable for anopheles. People of very low social status and living in very squalid conditions are, however, often found with little or no malaria, *e.g.*, Calcutta, Vizagapatam.

It is difficult to assign a value to racial apart from regional factors, as there is the association of aboriginal tribes and hill districts. The fact that a village of plains people also showed high infection seems to point to race not being the factor involved.

*Seasonal Variation.*—This is well illustrated by the examples from Mian Mir, Punjab. Here the supply of *A. culicifacies* is entirely from the irrigation canals. The variations in temperature are here extreme (from 120° F. in the shade during the hot weather to a minimum of 40° in December). Besides the effect of temperature in diminishing the number of larvæ, we have to allow for the fact that the canals are periodically shut off with a resulting marked decrease of larvæ.

It may be noted here that in the Syce lines (Table IV), though the most careful search was made, no anopheles, not even *A. Rossii*, could be found. We had here, therefore, a residual infection in the absence of anopheles, and with the onset of the cold weather a continued absence of anopheles and a still further reduction of the infection. The seasonal variation in the Punjab is unusually well marked.

Table III.—Race and Social Status.

Village.	Race.	Situation.	Parasite rate.	Spleen rate.	Remarks.
{ Sunku ..... Salur ..... Police lines ..... Bodawalasa ..... Kagowalasa ..... Kanai putti ..... }	Pariah, plains people ....	In hills within high endemic area	50	55	{ 15 miles apart only. Villages apparently do not contain aborigines. }
	Ditto .....	In plains in low endemic area	0	0	
	Plains people .....	In hills within high endemic area	50	91.6	
	Aboriginal tribe, Parasas	Ditto	86	57	
	Ditto, Gadobas .....	Ditto	50	63	
{ Village A ..... " B ..... " C ..... " D ..... Simachelam .... }	Ditto, Malis .....	Ditto	50	69	{ Variations dependent on local conditions. }
	Telugu, fisher caste. ....	Coast lands, Vizagapatam .....	0	0	
	Ditto .....	Ditto .....	10	17	
	Telugu, agricultural caste	Ditto .....	27	36	
	Ditto .....	Ditto .....	0	0	
{ Madras ..... Ennur ..... }	Telugu .....	In hills, 800 feet .....	..	25	{ Same race with different local conditions. }
	Tamils .....	In Blacktown .....	5	0	
	Ditto .....	Coast lands .....	54	95	



Table IV.—Showing Influence of Season in the Punjab (Mian Mir)  
(Cold and Dry Weather).

	Oct.	Nov.	Dec.	Remarks.
Inniskilling bazaar—				
Parasite rate.....	52	20	..	Breeding places still in existence.
Spleen rate .....	80	38	..	Larvæ and anopheles diminished in Nov. and Dec.
Royal Artillery bazaar—				
Parasite rate.....	35	..	29	Breeding places absent.
Spleen rate .....	75	..	34	
Sycc lines, 51st battery—				
Parasite rate.....	..	56	12	Scanty breeding places.
Spleen rate .....	..	48	30	
Cavalry lines—				
Parasite rate.....	..	24	..	Breeding places absent.
Spleen rate .....	..	36	11	

*Altitude.*—This of itself does not seem to play an important part under 4000 feet. In the mountain districts visited by us we have found two conditions.

1. Steep hill slopes intersected by mountain streams, which in the hot weather are frequently dry, and in the rains are rapid torrents. Under these conditions the facilities for breeding are reduced to a minimum, and in such cases malarial infection is almost nil. The Darjeeling tea gardens and Tonetta, near Mussoorie, N.W.P., are examples. (Table V.)

2. Small plateaus ensconced in the surrounding hills. Here there are numerous facilities for breeding in the swamps and rice fields, which are almost universally present under these conditions.

Sam Sing and Patingi are examples of this condition at a height of about 3000 feet. In these latter cases we find again often a high, endemic index.

*Regional Factor.*—While then in the individual portions of a particular district we are able to ascribe the variations found to different factors which may practically be resolved into one, viz., the abundance or scarcity of the infecting species of anopheles, yet there remains the more difficult question of why one region should be highly malarial and another not. It is difficult to explain what Celli has termed “paludismus sine malaria.” We have found parallel instances in India. In Bengal we have an area of low endemicity merging somewhat sharply into the highly malarious Duars. In Salur, Madras, we have innumerable *A. culicifacies*, with no enlarged spleens and no malarial infection, while 15 miles away at about 1000 feet the endemic index has already reached 50.

Table V.—Showing Relation between Altitude and Malarial Endemicity.

Village.	Altitude.	Parasite rate.	Spleen rate.	Anopheles and physical conditions.
{ Rungamutty Nagasuri Sam Sing }	feet.			
	800	43	83	Streams. Abundant <i>A. Christophersi</i> .
	1200	55	82	Streams. Abundant <i>A. Christophersi</i> and <i>A. maculata</i> .
{ Seypudura Lower Singell garden, Kurseong Upper Singell garden, Kurseong }	3000	28	7	Rice fields. Numerous <i>A. maculatus</i> .
	2000	0	12	Springs. Scanty <i>A. maculatus</i> .
	4000	0	5	<i>A. Rossi</i> . <i>A. maculatus</i> . Scanty. In huts.
{ Tonetta Rajpore }	4400	0	0	Swamp a few sq. yds. in area. <i>A. maculata</i> . <i>A. Lindseyi</i> . Scanty.
	6000	0	0	Spring. <i>A. Lindseyi</i> .
	3000	10	0	River bed. <i>A. maculatus</i> abundant.
{ Sunki Patingi Korapul Koomba }				
	2000	50	55	Stream. Abundant <i>A. culicifacies</i> and <i>A. maculatus</i> .
	2800	70	50	Marsh. Rice fields. Abundant <i>A. jeyporensis</i> .
	2000	20	70	Ditto.
{ Jeypore Hills Mandras. }	2000	60	100	Ditto.

Table VI.—Showing the “Regional Factor.”

Region.	Locality.	Parasite rate.	Spleen rate.	Common species of anophelos.
A region of low endemic index. Mueh swamp. Bengal	Caleutta .....	0	0	<i>A. Rossi.</i>
	Alipore .....	0	0	{ <i>A. Rossi.</i> <i>A. fuliginosus.</i> <i>A. sinensis.</i>
	Barrackpur .....	0	0	
	Belghuria .....	8	44	
	Ranaghat .....	14	0	{ <i>A. Christophersi.</i> <i>A. maculatus.</i>
	Jalpaiguri (1) .....	16	27	
A region of intense malarial endemicity (blackwater fever). The Duars	Jalpaiguri (2) .....	0	14	{ <i>A. Rossi.</i> <i>A. fuliginosus.</i> <i>A. sinensis.</i>
	” .....	0	0	
	Mainaguri .....	25	74	
	Rungamutty .....	43	83	{ <i>A. Christophersi.</i> <i>A. maculatus.</i>
	Nagasuri .....	55	82	
	Nagrakata .....	72	82	
A region of high endemic index. The Punjab	Enniskilling, B .....	52	80	{ <i>A. culicifacies.</i> <i>A. Rossi.</i>
	Royal Artillery, B .....	35	75	
	Syee lines, A .....	56	48	
	Syee lines, B .....	20	20	{ <i>A. culicifacies.</i> <i>A. fuliginosus.</i> <i>A. Stephens.</i>
	Nat. Cavalry lines .....	24	36	
	Phutala .....	45	50	
A region of not very intense endemic index, showing marked local variation. Central Provinces	Sakadara .....	15	15	{ <i>A. culicifacies.</i> <i>A. fuliginosus.</i> <i>A. Stephens.</i>
	Digdoh .....	20	25	
	Leendra .....	20	0	
	Ambajheri .....	25	5	{ <i>A. culicifacies.</i> <i>A. fuliginosus.</i> <i>A. Stephens.</i>
	Angni .....	25	40	
	Babulkiri .....	25	20	
	Takli .....	15	15	{ <i>A. culicifacies.</i> <i>A. fuliginosus.</i> <i>A. Stephens.</i>
	Telankeri .....	10	5	
	Kaamla .....	7	0	
	Sungeaon .....	8	0	{ <i>A. culicifacies.</i> <i>A. fuliginosus.</i> <i>A. Stephens.</i>
	Bighori .....	30	0	
	Lawa .....	5	0	
	Wandi .....	5	0	{ <i>A. culicifacies.</i> <i>A. fuliginosus.</i> <i>A. Stephens.</i>
	Wandi .....	5	0	

Region.	Locality.	Parasite rate.	Spleen rate.	Common species of anophels.
A region of low endemic index. Flat land near the coast. Northern Madras	Vizagapatam . . . . .	0	0	<i>A. culicifacies</i> .
	Tapara . . . . .	10	17	
	Chintaralli . . . . .	0	0	
	Fishing village. . . . .	27	36	
	Lower Simachelam . . . . .	5	10	
	Upper Simachelam . . . . .	..	25	<i>A. jeyporensis</i> (closely related to <i>A. Christophersi</i> ). <i>A. Listonni</i> (related to <i>A. Christophersi</i> ). <i>A. maculatus</i> .
	Salur (1). . . . .	0	0	
	" (2). . . . .	0	0	
	Sunki. . . . .	50	55	
	Police lines, Patingi . . . . .	50	92	
A region of intense malarial endemicity (and blackwater fever) sharply marked off from a low endemic region to the east. Jeypore Hill district	Patingi . . . . .	35	75	{ <i>A. culicifacies</i> . <i>A. Rossi</i> . <i>A. culicifacies</i> . <i>A. Rossi</i> .
	Bodawalasa . . . . .	86	57	
	Kanaiatty. . . . .	50	69	
	Kagowalasa . . . . .	50	63	
	Sisagoda . . . . .	70	50	
	Koraput . . . . .	20	70	{ <i>A. culicifacies</i> . <i>A. Rossi</i> . <i>A. culicifacies</i> . <i>A. Rossi</i> .
	Police lines, Koraput . . . . .	20	80	
	Koomba . . . . .	60	100	
	Ennur . . . . .	55	95	
	Madras . . . . .	5	0	
Area of intense malaria . . . . .				

Our observations have led us to conclude that a district may show a high endemic rate by reason of suitable local conditions (extensive breeding grounds close at hand) being repeated in the majority of the villages throughout the district. Were the physical conditions at Phutala reproduced in all the villages of the Nagpur district, we should get a district of fairly high endemic index.

The uniform presence of suitable local conditions is, however, quite insufficient to explain the data referring to regional variation. In regions of very high endemic index "dry" villages as well as "wet" villages exhibit extreme infection. In regions of low endemic index, the most suitable local conditions fail to give such a degree of infection as prevails in villages in intense malarial areas.

Phutala (Table I and Map), in the Central Provinces, though situated under conditions of the utmost suitability, fails to reach the rate of many "dry" villages in the Jeypore district or Duars. In very low endemic areas an abundance of anopheles seems incapable of giving rise often to any infection at all. In Bengal, villages surrounded by unlimited breeding grounds show extremely low endemic indices.

We have endeavoured to show that "species" is one determining factor. We have shown that *A. Rossii* is of little account in the spread of malaria, while certain other species are concerned in its transmission. How far variations in endemicity are due to this cause further work must show. Whether species of anopheles is the determining cause or not, we can say that over and above local conditions (the abundance of breeding grounds) there is another still more potent factor which we have called the "regional factor."

### Conclusions.

1. That other things being equal there is a direct relation between the extent and proximity of breeding grounds, the number of anopheles in the houses, and the endemic index.
2. That where breeding grounds are half a mile distant and have not recently existed closer, malaria is reduced to a minimum and anopheles are not to be found in the houses.
3. That the flight of anopheles (*A. culicifacies*, *A. fuliginosus*, and *A. Stephensii*) in Nagpur, Central Provinces, is frequently a quarter of a mile but does not extend to half a mile.
4. That the relation between the number of anopheles and endemic malaria is greatly modified by the species of anopheles present.
5. That *A. Rossii* does not carry infection under conditions in which *A. culicifacies* does.
6. That in India, areas of extremely high endemic index are found sharply separated off from areas of low endemicity.
7. That the distribution of endemic malaria depends, even more



than on local conditions in regard to breeding places, on the district concerned, and must at present be attributed to undefined causes which we have termed the "regional factor." The regional factor may be largely due to species but more accurate and detailed observations on the distribution of anopheles and malaria are necessary before this can be decided.

8. That in the two regions of intense malaria visited by us (Duars and Jeypore hills), blackwater fever was well known and has attacked a large proportion of the resident Europeans.

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"Note on Bodies in Salivary Glands of *Anopheles*," etc. By J. W. W. STEPHENS, M.D. Cantab., and S. R. CHRISTOPHERS, M.B. Vict. Received April 25, 1902.

[PLATE 5.]

(1.) Bodies which are probably encysted sporozoa were first found by us in *A. Rossii* at Jalpaiguri, Bengal. They have also been seen by us in a dissection of *A. fuliginosus*, Nagpur, C.P. Captain James, I.M.S., also reports finding them in *A. Rossii*, Ennur, Madras.

The following note was made by us:—" *A. Rossii*—one lobe of salivary gland is replaced by a mass of short sausage-shaped bodies (sporozoa). A few also in the substance of a second lobe. None found in the stomach." On fixing and staining by Romanowsky's method they show—

(1.) A definite capsule (red). The capsule is frequently frayed out or has a perforated appearance.

(2.) There are generally two chromatin bodies embedded in.

(3.) Cytoplasm (blue).

(4.) The size is roughly  $\frac{1}{3}$ — $\frac{1}{2}$  that of a red blood-cell. (*Vide* Plate 5, fig. 1.) Their characteristic shape is that of a sausage. They are quite different from the sporozoa previously noted by us in the ovaries of *A. Costalis*, which have also been figured by Celli; their nature is unknown to us. In the first instance they occurred actually in the gland substance; in the other instances they were free.

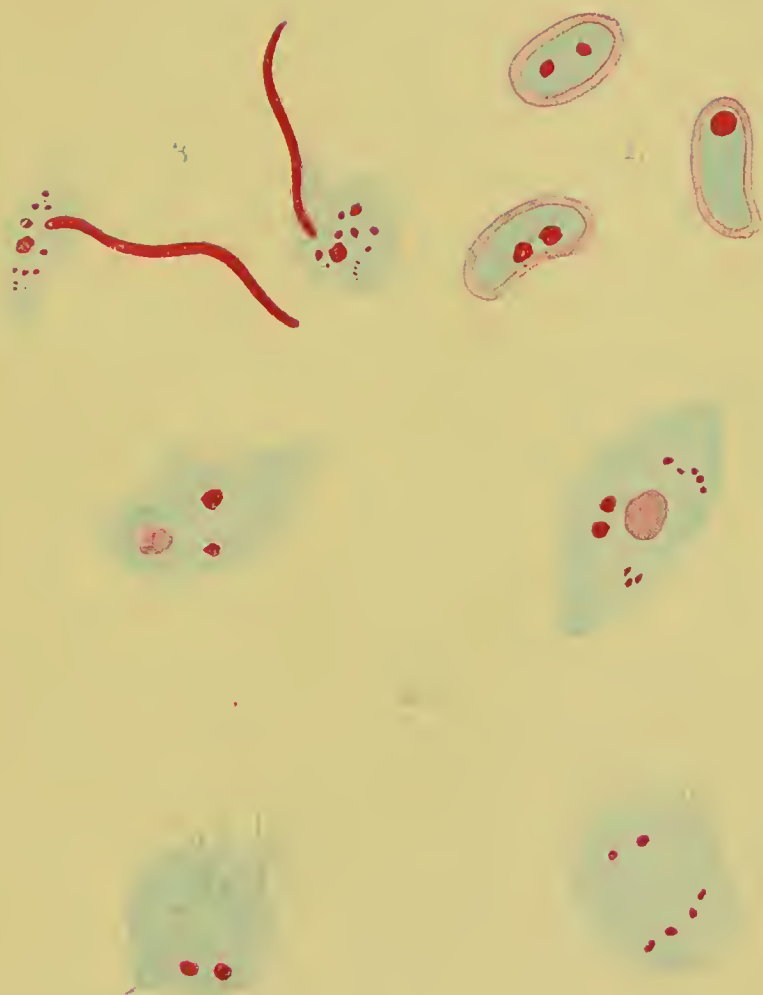
(2.) Bodies apparently identical with Ross's black spores. They were seen by us in a dissection of the glands of *A. fuliginosus* made by a Burmese prisoner at Nagpur jail. As they appeared in the dissection they had no connection with any cyst, nor were they in the

salivary gland itself. Their appearance did not suggest that they were sporozoa.

(3.) In dissecting the glands of anopheles from time to time, encysted bodies having apparently two sucking discs have been found by us in *A. Rossii* and in *A. fuliginosus*. They are probably the same as the bodies described by Italian observers as flukes.

(4.) Skin organisms—

- (a.) Very commonly in blood films made in Jalpaiguri, especially in the children of a particular school, bodies were found which had the following characters:—They are spherical or irregular in shape, and vary in size from  $7\ \mu$  to  $25\ \mu$ . With the Romanowsky's stain they show (1) one or more chromatic bodies of different sizes, (2) a reddish area, (3) a protoplasm staining blue or purple (hazy), (4) a distinct fringe of cilia is distributed all around or confined to one area. (*Vide* Plate 5, fig. 2.)
  - (b.) Bodies with a long and thick flagellum. They are pear-shaped or irregular. They show, with Romanowsky, chromatin particles. They occur commonly in certain districts as a skin contamination in blood films. (Plate 5, fig. 3.)
  - (c.) Very minute rings or discs about one-third the diameter of the youngest ring form of malaria parasite. Though a chromatic particle can be made out, the "vacuolic" nuclear area of the malaria parasite cannot be distinguished. They may occur on the blood-cell, but most frequently free. They have a flatter appearance than malaria parasites and are more minute. They disappear when great care is taken in cleansing the finger.
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“On a Convenient Terminology for the Various Stages of the Malaria Parasite.” By E. RAY LANKESTER, M.A., F.R.S., Director of the Natural History Department, British Museum. Received February 20,—Read March 6, 1902.

I have found it necessary in labelling a series of models of the malaria parasite in the Central Hall of the Natural History Museum to use as simple and clear a terminology as possible. I think that this terminology will be found useful by others who are perplexed by such terms as “sporozoites,” “blasts,” “ookinetes,” “schizonts,” “amphionts,” and “sporonts”—terms which have their place in schemes dealing with the general morphology and life-history of the group Sporozoa, but are not, as experience shows, well suited for immediate use in describing and referring to the stages of the malaria parasite.

It is necessary to treat the malaria parasite from the point of view of malaria; that is to say, to consider its significant phases to be those which it passes in the human blood. In reality its mature condition and most important motile, as well as its most prolific reproductive, phases are passed in the body of the mosquito.

1. The malaria-germ which is brought by the stab of the Anopheles into the human blood-vessels is a reproductive particle, a *spore*. It is needle-like in shape, and might be named in reference to its form (*e.g.*, oxyspore or raphidiospore), but the most important fact about it for description and comparison is that it has been formed *outside* the human body, and is introduced as a strange element into the human blood by the agency of the mosquito. I therefore call it the EXOTOSPORE.

2. The Exotospores (probably as many at a time as forty or fifty) enter the blood by the agency of the mosquito's stab and immediately penetrate, each one, a red corpuscle. The history of this process has not been observed. As soon as it has entered a red corpuscle the exotospore loses its needle-like shape and becomes amœbiform. I apply to it the name I proposed some years ago for similar amœbiform spores in other Protozoa, namely, AMŒBULA.\*

3. The Amœbula exhibits amœboid movements within the red corpuscle, enlarges and finally breaks up into spherical spores, which are liberated with destruction of the red corpuscle. It seems to me unnecessary to have a special name for the star-like or other condition of the Amœbula when in course of breaking up into spores; but the spores so produced require a special name which shall emphatically distinguish them from the Exotospores. I call them the ENHÆMOSPORES, in reference to the fact that they are produced by a

\* ‘*Encycl. Britann.*,’ Article “Protozoa.”



process of division which occurs *in* the blood of the malaria-stricken human being.

4. The *Enhæmospores* penetrate fresh red blood-corpuscles, and after a certain growth as *amoebulae* break up into a new crop of *Enhæmospores*, by which the infection of the red corpuscles is extended. This process appears to go on for several generations and for a varying duration of time. But owing to conditions and at a period of the infection which has not been precisely ascertained, some (or all ?) of the *amoebulae* derived from *Enhæmospores* cease to break up into spores. Instead of carrying out that process they enlarge, and in the case of the *æstivo-autumnal* parasite (*Laverania præcox*) become sausage-shaped or, as it has been termed, crescent-shaped. This change of form is accompanied by a destruction of the red corpuscle and the formation of granules of dark pigment within the parasite. It seems best to term this phase the "CRESCENT" or "CRESCENT-SPHERE," the latter term being applicable to those species in which the form is not markedly *erescentic*.

5. The crescents or crescent-spheres remain quiescent in the human blood. They are, however, of two different natures—male and female. It is not possible to distinguish with any certainty the male from the female crescents whilst they remain in the human blood-vessels. But it is these bodies which are destined to be swallowed by the *Anopheles* mosquito, and to carry on further the life-history of the parasite.

The crescents are therefore the sexual phase of the parasite. When the crescents are swallowed by a mosquito (of an appropriate species), they undergo two different modes of development, determined by the fact of their sex. Both sexes become spherical, and may now be called respectively "EGG-CELL," and "SPERM-MOTHER-CELL."

From the periphery of the SPERM-MOTHER-CELL, now floating in the mosquito's stomach, there are developed with surprising rapidity six or seven SPERMATOZOA, which for a time remain attached to the residual mass (or SPERM-BLASTOPHOR) of the sperm-mother-cell. Complete cytological study of this development is still wanting, but it appears that the spermatozoa are true spermatozoa, like those of the higher animals, and have the same relation to the mother-cell from which they develop as is the case in such an animal as the Earth-worm.

The EGG-CELL, now also floating in the mosquito's stomach, apparently gives rise to one, and possibly to two, polar bodies, but the observations on this point are, as yet, insufficient.

Fertilisation of the egg-cell now takes place in the gnat's stomach. A single spermatozoon penetrates and fuses with each egg-cell.

The fertilised egg-cell is spoken of as a "zygote"; it is also described as the sexually produced embryo.

6. The ZYGOTE or SEXUALLY PRODUCED EMBRYO remains unicellular, but increases in size and becomes pyriform. It exhibits active move-

ments of expansion and contraction in the line of its long axis, and also a quick movement of its narrower end alternately to either side. This is the largest growth of the individual cell attained to in the series presented by the life-history of the malaria parasite. It has been called "vermiform" and "vermicule" (Ross), and I adopt this name for it, viz., the VERMICULE. The vermicule is the dominant individual form in the history of the malaria parasite, endowed with greater size, power, and activity than other phases. It corresponds, not only in this respect, but also in its position in the life cycle, to the large often active cells of the Gregarinidea, which I proposed some time ago to call the Euglena-phase.\*

It is worthy of note that in the size and activity of the Vermicule, the Hæmaosporidia—the order of Sporozoa which embraces the malaria parasite—come nearer to the Gregarinidea than they do to the Coccidiidea, though in the existence of a sexual generation absent in Gregarinidea, they agree with the Coccidiidea.†

The vermicule now pushes its way through the tissues of the gnat's stomach and in the blood sinuses outside the stomach becomes spherical. It enlarges and nourishes itself on the insect's blood, and forms a spherical CYST, or structureless transparent envelope. This cyst is destined to enlarge, with vast increase of its living contents.

The living cell within the cyst breaks up by a definite process to form eventually an immense number of exotospores, the stage with which the present description commenced. The CYST would most conveniently be called a "sporocyst," since, as so often happens in Protozoa, it is formed purely and simply in relation to the quiescence of the organism and its division into numerous reproductive spores. Unfortunately, the word "sporocyst" has been employed recently by writers on the Sporozoa for the small capsules containing one or two to eight elongated spores which used to be called "pseudonaviculæ," and are formed *within* such larger cysts as that now in question. The word "cyst" should have been reserved for the larger more general protective envelope, and the "pseudonaviculæ" might have been called "sporo-thekæ." In any case, I think we may call the cysts in which the vermicules of the malaria parasite enclose themselves "SPORE-CYSTS" or "SPORE-FORMING CYSTS." The name "oocyst," applied to them by some writers, is simply misleading.

7. The spore-cysts lying outside the stomach wall of the mosquito bathed in the insect's blood receive abundant nourishment. The single-celled vermicule enclosed undergoes rapid changes; it increases greatly

\* 'Encycl. Britann.,' Article "Protozoa."

† A sexual phase has been described in the Gregarine *Stylorhynchus* by Léger since this paper was written. It occurs at an unexpected point in the cycle: two encysted full grown "Sporonts" are stated to produce the one egg-cells the other spermatozooids!

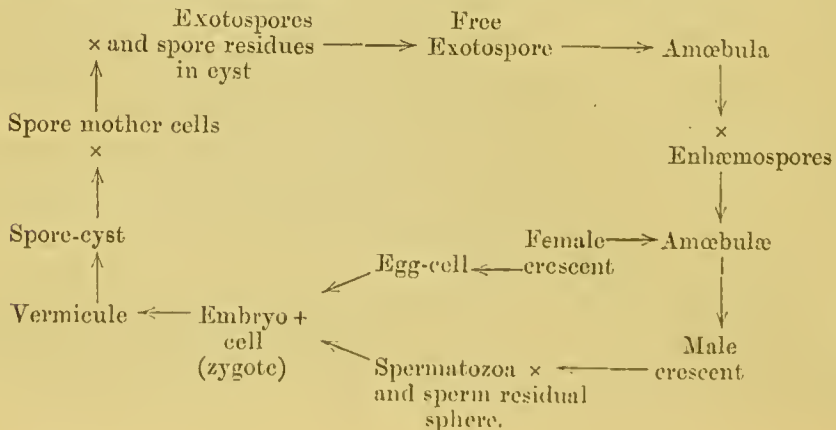
in volume and breaks up by normal cell division (? the earliest steps have yet to be studied) into a number of SPORE-MOTHER-CELLS. In the process of this division and the later stages of the final development of the "spores" (exotospores), the "spore-forming cyst" increases in size to twenty times its initial diameter.

The spore-mother-cells are set closely together in the cyst; they are of polygonal shape, owing to pressure, and each has its nucleus. Finally they give rise, each spore-mother-cell, to a crop of filiform spores (exotospores) which have the same relation to the spore-mother-cell as spermatozoa have to a sperm-mother-cell, viz., they form on the outside of the spore-mother-cell as outstanding processes, carrying away all the chromatin of the mother-cell and leaving in the centre or to one side a "residuary body," a "spore blastophore" similar to the "sperm-blastophore" of spermatozoon-development.

Thus we are brought back to the needle-like exotospores with which we started.

The spore-holding cysts burst and liberate the exotospores into the blood of the mosquito. Thence they readily pass into the ducts of the salivary gland, and so are conveyed by the mosquito's stabbing beak into human beings. A point in this connection is the definite ejection by the mosquito of the secretion of its salivary gland into the punctured wound which it makes in the human skin. There can be no doubt that such an ejection takes place. The leech ejects a secretion on to the wound caused by its bite which has the property of preventing the coagulation of the blood. It is possible that the mosquito and other blood-sucking flies may use the salivary secretion for the same purpose. It is obvious that unless there were some injection into the wound on the part of the fly, the chances of infection of the bitten animal by the parasites carried by mosquitoes or tsetse fly would be very small.

Our cycle of forms with the names here made use of may be written as below. The sign  $\times$  is used to indicate fissile multiplication, and  $+$  to indicate fusion, while  $\rightarrow$  merely indicates continuity.





Malaria.		Coccidium.		Gregarina.
1.	Exospore, free in human blood ("Blast" of some authors.)	Sporozoite	Sporozoite	Sporozoite. (Filiform young.)
2.	Amœbula, in red corpuscles	Schizont	Amœbula	Amœbula.
3.	Enhæmospore, ditto, and in blood	Merozoites, formed by schizogony		
4.	Crescent, in human blood.	Gametocytes		
	a. Male	Microgametocyte		
	b. Female	Macrogamete		
5.	Sperm-mother-cell, in gnat's stomach	Microgametocyte		
6.	Egg-cell, in gnat's stomach	Macrogamete		
7.	Spermatozoon, in gnat's stomach	Microgamete		
8.	Zygote or embryo-cell, in gnat's stomach.	Young oocyst (sporont)		
9.	Vermicule, in gnat's stomach	WANTING		
		(Called "ookinete" or "kineto-sporont" in the nomenclature of this column.)		Full-grown motile "gregarine." (Euglenoid phase.)
10.	Spore-cyst, in blood-sinus outside gnat's stomach	Older (but not larger) oocyst or sporont		Cyst enclosing one or two full-grown sporonts.
11.	Spore-mother-cells in cyst, in blood-sinus outside gnat's stomach	Sporoblasts (sporogony)		Sporoblasts. (? Conjugation in <i>Lankesteria</i> <i>Ascidia</i> . Spermatozoa and ova in Stylorhynchus.)
12.	Exotospores in cyst, in blood-sinus outside gnat's stomach	Sporozoites enclosed in small groups in sporocysts within the bigger oocyst.		Sporozoites enclosed in capsules, called "pseudonaviculae" or "sporocysts."
21.	Free exotospores, in gnat's salivary duct	Free sporozoite		Free sporozoite.

Schizogony rare: sexual stages NOT  
OBSERVED and probably WANTING.

I also give a list of the names here used with reference to the occurrence of the forms indicated in man or in gnat and an indication of the corresponding stages in a Gregarina and a Coccidium. In the column belonging to coccidium I have employed the generalised physiological nomenclature accepted by special students of the Sporozoa (Schaudin, Lühe, &c.)

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